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A BEGONIA IMMUNE TO CROWNGALL: WITH OBSERVATIONS ON OTHER IMMUNE OR SEMI-IMMUNE PLANTS

(The first of a series of Potentiometer Studies.)

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On the crowns of a tall-growing begonia, long cultivated in our houses on account of its large flower clusters and showy foliage, we observed numerous natural galls supposed to be crowngalls. When examined more critically, they were found to be nematode galls due to *Heterodera radicicola*. Subsequently, inoculations of virulent cultures of the hop strain of *Bacterium tumefaciens*, on a large scale, three times repeated, failed to produce any tumors on these plants, although the same cultures inoculated into sunflowers, sugar beets and *Ricinus* developed the usual tumors within the ordinary time (Fig. 2).

This plant, known to us as *Begonia lucerna*, said to be one of the derivatives of *B. coccinea* (*B. rubra* of gardeners), has large, very oblique, smooth leaves which are very red on the under-surface, especially the young leaves, and green above with silver spots (Fig. 1). With plenty of room the plant often grows to a height of 6 or 8 feet or more, and the red flower clusters may be a foot long and equally broad.

The above-mentioned unexpected results led to the following potentiometer tests and other tests. In each case we experimented with 10 cc. of undiluted juice, which was tested in a Hildebrand calomel-cell apparatus as soon as possible after its extraction. The plants were ground in a meat chopper, and the juice was obtained by means of a small hand press. Whenever minutes are used in connection with hydrogen, it means minutes from the beginning of the grinding to the beginning of the hydrogen saturation of the platinized needle. T. = Temperature in degrees Centigrade; mv. = millivolts; and T.a. = Total acidity in terms of Fuller's scale, *i.e.*, number of cubic centimeters of $\frac{N}{1}$ NaOH required per liter of juice to bring the fluid from the given pH to pH 8.2 (phenolphthalein neutrality). In each

FIG. 1. Leaf of *Begonia lucerna*, 7/8 natural size, showing the numerous silver spots.

case the readings on the potentiometer were continued until there was no further rise in potential, and the highest reading was always taken for computing the pH by means of the constants of Michaelis.

Exp. 157. March 20, 1924, 10:30 A. M. Removed and ground 50 large upper leaves from numerous shoots. None flabby or yellowed. Weight 175 grams. With moderate pressure we obtained about 100 cc. of a strongly acid, non-viscid, opaque fluid, the color of capillary blood. Hydrogen on in 18 min. T. 26; mv. 324.2; pH 1.35; T.a. + 247 (estimated; too much alkali was added at the end). This result, only approximately true with respect to total acidity, was so astonishing that the experiment was immediately repeated, with the following result.

Exp. 158. The acid fluid was centrifuged and we obtained a clear blood red fluid which tasted a little like currant juice, *i.e.*, it was strongly acid and somewhat acerb. Hydrogen on in 115 min. T. 26; mv. 325.5; pH 1.36; T.a. + 263.

Exp. 159. The soft green tops of 40 shoots of *Begonia lucerna* were ground after removal of the leaves. Weight 333 grams. From these under moderate pressure we obtained 200 cc. of a cloudy, dirty, yellowish green, non-viscid fluid containing only a trace of pink color. After centrifuging, the fluid was still cloudy and of a pinkish yellow color. Hydrogen on in 25 min. T. 25; mv. 377.8; pH 2.23; T.a. + 99.

Exp. 160. Bases of 76 shoots taken from about a dozen pots. Nine-tenths of these shoots were covered with a smooth brown-purple bark, the rest were green on the surface, a few were rather woody. All of these plants had nematode tumors at the surface of the earth and below. Weight 390 grams. Extracted 210 cc. of a yellowish green fluid, which was still cloudy and yellowish green after centrifuging. Hydrogen on in 32 min. T. 25; mv. 447.8; pH 3.42; T.a. + 40.5.

Exp. 161. Nematode tumors from the bases of 36 stems. All small, considerable necrosis in each one. Many others were too badly decayed to use. We obtained only a small double handful of usable galls, few of which were perfectly sound. Extracted about 60 cc. of a dirty, yellowish-white, very cloudy fluid from which a muddy brown precipitate settled out at once. On centrifuging there was an additional dirty precipitate containing a little starch, but the fluid remained thinly clouded and whitish. Hydrogen on in 18 min. T. 26; mv. 458.4; pH 3.60; T.a. + 32.

Somewhere between pH 4.28 and pH 4.80 in the titration curve (sodium hydroxide being added) the color of this tumor juice changed from white to blue, but at the end of the titration (pH 8.2) it was dark red. This result appeared so inexplicable that the experiment was repeated to see if the hastily recorded first dark color (blue) was not an erroneous determination.

Exp. 162. Into 10 cc. of the white fluid of Exp. 161 we allowed $\frac{N}{20}$ NaOH

to stream slowly. At first the thin stream of clear alkali looked like a smoke cloud as it entered the juice. When enough had been added, the

FIG. 2. Left: Shoots of *Begonia lucerna* (fourth series) inoculated copiously (20 needle pricks) with *Bact. tumefaciens*, hop strain. Right: two controls on *Ricinus* (inoculated from the same tube). Time six weeks (nearly). The whitish patches on the begonia shoots are masses of bacteria through which the punctures were made. 2/3 nat. size.

fluid became a pale blue; with further additions of alkali the fluid became first a deep blue, then purple, and finally a deep red.

Exp. 163. We now took the juice of Exp. 160 (base of the shoots) and allowed the dilute alkali to stream into it in the same way. After a time it also became pale blue, then deep blue and finally red as in Exp. 161 and 162.

Exp. 164. The test was now repeated on juice from the top of the shoots (Exp. 159), with the same result.

Exp. 165. A final test was made on 10 cc. of the centrifuged blood red juice from the leaves (Exp. 158). On addition of $\frac{N}{1}$ NaOH the clear, blood red fluid apparently behaved differently (see later), the first observed change being a purple-color, then brown, then darker brown, then dirty bluish, and finally greenish. We continued to add alkali but with no production of any final red color. It stayed in the green.

The following year, 1925, the juice of leaves and stems of this plant was extracted, centrifuged, titrated electrometrically with sodium hydroxide down to a pH within the limits of growth of *Bact. tumefaciens* (its bouillon range as determined in this laboratory is approximately pH 5.70 to pH 9.20), sterilized, retested in order to determine that the titre had not changed, and inoculated with the hop strain of *Bact. tumefaciens*. In the juice thus treated the crowngall organism grew readily. One of these juices registered pH 6.56, the other pH 7.10, and the best growth was in the latter. In the third set, to which an excess of alkali had been added, there was a very copious precipitate on steaming, leaving a colorless fluid (the others were dark) the final titre of which was decidedly more acid (pH 4.55) than before the steaming; and in this fluid, as we expected, the organism would not grow. Before adding alkali the acidity of these juices as determined on the potentiometer was as follows:

Exp. 521. Blood red juice from the upper leaves, pH 1.06.

Exp. 522. Pale juice from soft tops of shoots, pH 1.33.

On adding clear lime water to the acid juice a very copious precipitate of insoluble calcium oxalate was obtained, *i.e.*, the washed white precipitate was insoluble in oxalic acid or acetic acid, but readily soluble in dilute hydrochloric acid. A part of the acid, therefore, is oxalic acid, but not all of it, since lime salts remained in solution in the fluid, as shown by evaporating some of it.

Some weeks later, August 18, 1925, a third set of tests was made on the leaf, stem, and nematode tumor juices of *Begonia lucerna*. At 11:15 A. M. we cut 21 shoots, which were brought into the laboratory and tested with the following results:

Exp. 537. From the 21 shoots we removed 64 upper leaves and commenced to grind them at 11:30 A. M. Extracted 190 cc. of red, acid juice.

Centrifuged 10 minutes. Fluid a clear blood red. Hydrogen on in 65 min. Fluid not viscid. T. 29; mv. 323.5; pH 1.34; T.a. (lost)—too much alkali was added at the end, *i.e.*, 0.5 cc. of $\frac{N}{1}$ NaOH carried the record from mv. 640.5 (pH 6.64) to mv. 912.5 (= pH 11, color then brownish green), the desired reading for pH 8.2 being mv. 733.78. T.a. beyond +250.

Exp. 538. Duplicate of Exp. 537. Hydrogen on in 90 min. T. 29; mv. 313.0; pH 1.31; T.a. +264.

Some preliminary tests were made on the juice from these leaves by slowly adding $\frac{N}{20}$ NaOH. The first color (on the border line of the two fluids) was a fugitive blue, overlooked when the stronger alkali was used in 1924 (Exp. 165), then purple, then brown, becoming, with more and stronger alkali $\left(\frac{N}{1}\right)$, yellowish green.

Exp. 539. Commenced to grind the 21 soft green shoots at 2 P. M. Extracted 245 cc. of a turbid yellow fluid. Centrifuged 10 min. Fluid a clear salmon color (yellowish pink). Hydrogen on in 48 min. T. 30; mv. 349.8; pH 1.79; T.a. +118. At mv. 505.7 (pH. 4.39) there was a slight change in color which deepened at mv. 518 and became definitely blue at mv. 554 (pH 5.19). At mv. 622 (pH. 6.32) the fluid was a deep violet blue. At mv. 663.6, reddish blue (Quirk), purple red (E. F. S.). At mv. 911 (pH 11) this fluid was brown red, whereas the juice from the leaves was brown green (see Exp. 537).

Exp. 540. Tested a double handful of nematode galls collected in fresher condition than those of 1924. Commenced to grind at 4 P. M. Obtained 110 cc. of a yellow milky juice. Centrifuged 10 minutes. A copious dirty precipitate and a translucent milky yellowish fluid. Hydrogen on in 30 min. Fluid not viscid. T. 30; mv. 426.8; pH 3.07; T.a. +54. At mv. 523 (pH 4.68) the first distinct pale blue color; at mv. 563.5 (pH 5.36) the fluid became deep blue; at mv. 608.5 it was purplish; at mv. 631, reddish; from mv. 649 on, the fluid was red.

The following additional tests were made on August 31, 1925.

Exp. 544. Soft tops of 24 shoots (leaves removed). Cut at 11:50 A. M. Commenced to grind at 12:10 P. M. With moderate pressure we obtained 182 cc. of an opaque, olivaceous juice. Centrifuged 10 min., fluid a clear pink. Hydrogen on in 27 min. T. 29; mv. 350.2; pH 1.79; T.a. +135 (estimated; too much alkali was added at the end). No change in color from mv. 350 (pH 1.79) to mv. 465 (pH 3.71); at mv. 476 (pH 3.90) a trifling change toward blue; at mv. 506 (pH 4.40) still pink; at mv. 532 (pH 4.83) the neutral point not yet reached; between this pH and mv. 613.8 (pH. 6.19) the preceding color changed to blue; at the end point (pH 8.2) it was red.

FIG. 3. Inoculated slow-growing crowngalls on the B. P. I. hybrid begonia and leaf of the same. Juice pH 1.65. Time 4½ months. Photo. Jan. 20, 1926, 7/8 natural size.

Exp. 545. Duplicate of Exp. 544. Hydrogen on in 65 min. T. 30; mv. 350.2; pH 1.79; T.a. + 140. At mv. 584 (pH 5.69) pale blue; at mv. 606.6 (pH 6.07) deep blue; at mv. 635.7 (pH 6.55) violet blue; at mv. 670.7 (pH 7.14) purple blue; at mv. 686.5 (pH 7.40) purple red; between mv. 722.6 and mv. 742.0 (pH 8.00 to 8.32) red.

Exp. 546. Bases of 24 old stems (9 only with brown bark). Commenced to grind at 2:00 P. M. Extracted 225 cc. of opaque sulfur-green juice. Centrifuged 10 min. Fluid still opaque and yellow. Hydrogen on in 49 min. T. 31; mv. 441.5; pH 3.30; T.a. + 56. At mv. 540 (pH 4.84 the fluid changed to blue, at mv. 586 (pH 5.72) it was a deeper blue; at mv. 636.7 (pH 6.57) it was violet blue; at mv. 698 (pH 7.58) it was red purple; and at mv. 739.7 (pH 8.28) it was red.

Exp. 547. Duplicate of Exp. 546. Hydrogen on in 80 min. T. 31; mv. 440; pH 3.30; T.a. + 56. Color bluish at mv. 532 (pH 4.83); deep blue at mv. 574.7 (pH 5.54); deep blue at mv. 653 (pH 6.84); red at mv. 714 (pH 7.85) and onward to mv. 731 (pH 8.11).

TABLE 1.—*The H-ion concentration and total acidity of the juice of Begonia lucerna*

Experiment No.	Degrees Centigrade	Millivolts	pH	Total acidity (undissociated salts)
Leaves				
157	26	324.2	1.35	+ 247 estimated
158	26	325.5	1.36	+ 263
521	.....	.....	1.06	.....
537	29	323.5	1.34	Lost (250 plus)
538	29	313.0	1.31	+ 264
548	30	296.0	0.90	+ 250
Top of shoots				
159	25	377.8	2.23	+ 99
522	.....	.....	1.33	.....
538	30	349.8	1.79	+ 118
544	29	350.2	1.79	+ 135 (estimated)
545	30	350.2	1.79	+ 140
Base of shoots				
160	25	447.8	3.42	+ 40.5
546	31	441.5	3.30	+ 56
547	31	440.0	3.30	+ 56
Nematode tumors on crowns				
161	26	458.4	3.60	+ 32 (somewhat decayed)
540	30	426.8	3.07	+ 54
600	24	458.5	3.60	+ 35
601	24	458.6	3.67	+ 35

TABLE 2.—*Behavior of Bact. tumefaciens (hop strain) in a graded series of peptone beef bouillons, inoc. Aug. 28, 1925. First examination at end of 24 hours at 25° C.*

pH (colorimetric)	Behavior		pH of controls on Sept. 2 (potentiometer)
	Aug. 29	Both clear	
5.2	do 31	do	
	Sept. 2	do ck. clear	.....
	Aug. 29	Both clear	
5.4	do 31	do	
	Sept. 2	do ck. do	.....
	Aug. 29	Both clear	
5.5	do 31	do	
	Sept. 2	do ck. do	.....
	Aug. 29	Both clear	
5.6	do 31	do	
	Sept. 2	do ck. do (uncorrected bouillon)	5.6
5.8	Aug. 29	One cloudy, one clear. The cloudy tube received the most inoculum	
	do 31	One cloudy, one clear	
	Sept. 2	Both cloudy with a good pellicle, ck. clear	5.7
	Aug. 29	Both cloudy	
6.1	do 31	Cloudier with pellicle	
	Sept. 2	Both well clouded with good pellicle, ck. clear	.....
	Aug. 29	Both cloudy with flocculence	
6.3	do 31	Better growth. Pellicle	
	Sept. 2	Both well clouded. Good pellicle, ck. clear	.....
	Aug. 29	Best growth. Well clouded, thin pellicle	
	do 31	do do. good do	
7.0	Sept. 2	Heavy pellicle in each tube. Fluid beginning to clear, ck. clear	.....
	Aug. 29	Inoc. to-day from pH 7.0 of Aug. 28	
	do 31	Both cloudy	
8.6	Sept. 2	Each well clouded with pellicle. Less clouded now than in pH 6.3, ck. clear	.....
9.0	Aug. 29	Both cloudy with plain rim breaking up on shaking	
	do 31	Heavier growth with pellicle	
	Sept. 2	Moderate clouding, good pellicle, ck. clear	.....
	Aug. 29	One tube thinly clouded, one clear	
	do 31	Both clouded with pellicle	
9.4	Sept. 2	Good pellicle, twice as much clouding as in pH 9.8, ck. clear	8.84
	Aug. 29	Both clear	
9.8	do 31	do	
	Sept. 2	Both clouded with a thin pellicle, ck. clear	9.05

In March, 1926, another test was made of juice from nematode galls on this begonia with the following results:

Exp. 600. Hardly any starch in the precipitate, *i.e.*, not more than 1/30 of the whole. Fluid slightly cloudy and pale yellowish. Hydrogen on in 33 min. T. 24; mv. 458.5; pH 3.60; T.a. + 35.

Exp. 601. Duplicate. Hydrogen on in 59 min. T. 24; mv. 458.6; pH 3.67; T.a. + 35. At pH 4.50 the fluid was still yellow; at pH 5.48 it was blue; at pH 7.00 it was violet red; and at pH 7.53 and on, it was red.

For the convenience of the reader the potentiometer determinations are assembled in table 1.

Up to this time we had data on the behavior of *Bact. tumefaciens* in acid media only, as given by Quirk and Fawcett.[1] Those data leave a gap between pH 5.7, at which the organism grew, and pH 5.2, at which no growth was obtained. To fill this gap and also to redetermine its behavior in alkaline bouillons we prepared a series of graded peptone beef infusion bouillons and inoculated them immediately after the pH tests were made. The inoculum was, in each case, a clearly visible small quantity of bacterial growth from an 8-day agar streak (all from the same tube) carried on the tip of a platinum needle. Two tubes of each grade were inoculated and a third was held as a control. The results, confirmatory of the earlier observations, are given in table 2.

Some change toward the acid side allowing growth might have occurred over night in pH 9.4, before the clouding was observed on Aug. 29, as the strongly alkaline beef bouillons do not remain very long where they are put.

On Sept. 2 at 4 P. M. four additional alkaline bouillons were tested on the potentiometer and immediately inoculated. Temp. 25° C. Two cks. held

TABLE 3.—*The reaction of Bact. tumefaciens in alkaline peptone bouillons*

pH	Behavior	
9.00	Sept. 3	10 A. M. One thinly clouded, one clear (that receiving the the least inoculum).
		4 P. M. Both thinly clouded, no rim or pellicle.
	do 4 and 5	Both thinly clouded, rolling clouds on shaking, trace of rim.
	do 8	Good pellicle on each one. Checks clear.
9.19	Sept. 3 and 4	Both clear.
	do 5	3 P. M. Both thinly clouded.
	do 8	Cloudier than on Sept. 5, but thin. Both checks clear.
9.41	Sept. 3 to 9	Both clear. Checks clear.
9.65	Sept. 3 to 9	Both clear. Checks clear.

[1] Quirk and Fawcett. Hydrogen-ion concentration vs. titratable acidity in culture mediums. Jour. Inf. Diseases 33: 48. 1923.

on each pH and two tubes inoculated, one much heavier than the other. Each received a 3 mm. loop of inoculum from bouillon pH 6.3 (preceding series), and one also a good sized fragment of pellicle, *i.e.*, 50 to 100 times as many bacteria as the other. The results are given in table 3.

EXPERIMENTS ON OTHER BEGONIAS

The foregoing experiments led to tests of several other begonias which likewise were found to be very acid but not entirely resistant to crowngall inoculations.

The Phyllomaniac Begonia

Exp. 532. Aug. 10, 1925, 11:10 A. M. Brought in 46 young shoots of *Begonia phyllomaniaca* and removed the leaves. Weight of the shoots 512 grams. Commenced to grind at 11:40 A. M. Extracted under moderate pressure 390 cc. of a muddy green fluid. The first taste was strongly acid followed by a bitter taste like quinine or quassia. Hydrogen on in 45 min. T. 30; mv. 358.5; pH 1.94; T.a. lost (too much alkali added at the end). The proper record was somewhere just beyond + 150.

Exp. 533. Duplicate of Exp. 532. Hydrogen on in 127 min. T. 30; mv. 356.9; pH 1.91; T.a. + 153. At mv. 506 (pH 4.35) the pink color disappeared, and at mv. 596 (pH 5.83) the fluid became blue.

Exp. 534. Juice of about 300 leaves from the 46 shoots. They had been kept closely wrapped and were not wilted. Commenced to grind at 2 P. M. Extracted 470 cc. of a muddy chocolate brown fluid. Centrifuged 10 minutes. The fluid was now cinnamon color and still opaque. It was centrifuged another 10 min. (3,000 rev. per min.) without change of color or opacity. Hydrogen on in 69 min. T. 31; mv. 333.0; pH 1.52; T.a. + 206.

On adding 250 cc. of lime water to an equal volume of the stem juice, we obtained evidence of oxalic acid, *i.e.*, an immediate copious white precipitate soluble in dilute HCl but insoluble in oxalic acid or glacial acetic acid. The leaf juice treated in the same way became milky cloudy but did not precipitate for a long time, not even on adding another 150 cc. of the lime water and finally 240 cc. more. On centrifuging, the fluid partly cleared, yielding a dirty white precipitate, part of which dissolved in HCl.

We had no expectation of obtaining crowngalls on these plants, but inoculations from agar streaks on September 3, 1925, made in the soft upper stems of about 20 of these plants with the hop strain of *Bact. tumefaciens*, produced slow-growing tumors on most of them. Those that failed to produce tumors were plants that grew poorly, and rotted off at the base a little later from overwatering and the attack of *Fusarium*. These tumors were small and thickly studded with shoots (Fig. 4B).[2]

[2] For numerous photographs and observations on this strange plant see Bacterial Diseases of Plants, pp. 574–621. W. B. Saunders Co., Phila. and London, 1920.

The Vernon Begonia

The following experiments were made with the Vernon begonia, a favorite bedding plant on account of the profusion of rose red to rose pink flowers (R_2). The leaf blades, seldom more than 2 to 2½ inches broad and not much longer, are fine, doubly serrate and very oblique (Fig. 5). They are a glossy metallic green above and a dull green below with traces of red, petioles pink. The stems are greenish or reddish. Through a hand lens the under surface of the leaf blade shows innumerable small roundish white spots, which, under the compound microscope, are seen to be islands of stomata. The plant is smooth except for scattered hairs.

Exp. 544. Ground 20 fresh shoots of the Vernon begonia (leaves removed). Extracted 130 cc. of an opaque cinnamon red juice. Centrifuged 10 min. Fluid now a clear bright red. Owing to an accident the juice was set into the icebox over night, and the following tests were made the next morning.

Exp. 548. T. 29; mv. 340.8; pH 1.63; T.a. ————.

Exp. 549. Duplicate of Exp. 548. T. 29; mv. 340.8; pH 1.63; T.a. + 191.

Two other tests of the total acidity gave + 193 and + 194.

Here again, from the results on *Begonia lucerna*, we had no expectation of getting any tumors from needle pricks introducing *Bact. tumefaciens* (hop strain), but after some months a few small tumors developed (Fig. 4C).

B. P. I. Hybrid Begonia

On September 3, 1925, crowngall inoculations were also made on the soft upper part of four plants from a hybrid begonia of uncertain parentage. It was produced in the Bureau of Plant Industry, but we have not learned who made the cross. Only these four plants were available for the experiment. No positive results were expected, but slow-growing tumors developed on each one of the four plants (Fig. 3). The plants continued to grow beyond the point of inoculations, and potentiometer tests of the tops of these plants made on January 20, 1926, gave the following results.

(1) Ground upper stem of the 4 plants, *i.e.*, above the tumors (leaves removed), and extracted 55 cc. of opaque cinnamon-colored juice. On centrifuging, a little starch and chlorophyll were thrown down and a clear scarlet red acid fluid remained with the following reactions: T. 25; mv. 341.8; pH 1.62; T.a. + 156, approximate. Duplicate, T. 25; mv. 342.6; pH 1.60; T.a. + 154. Second dupl., T. 25; mv. 343.6; pH 1.65; T.a. + 153.

(2) Forty-eight leaves of the same. T. 25; mv. 342.8; pH 1.66; T.a. + 160.

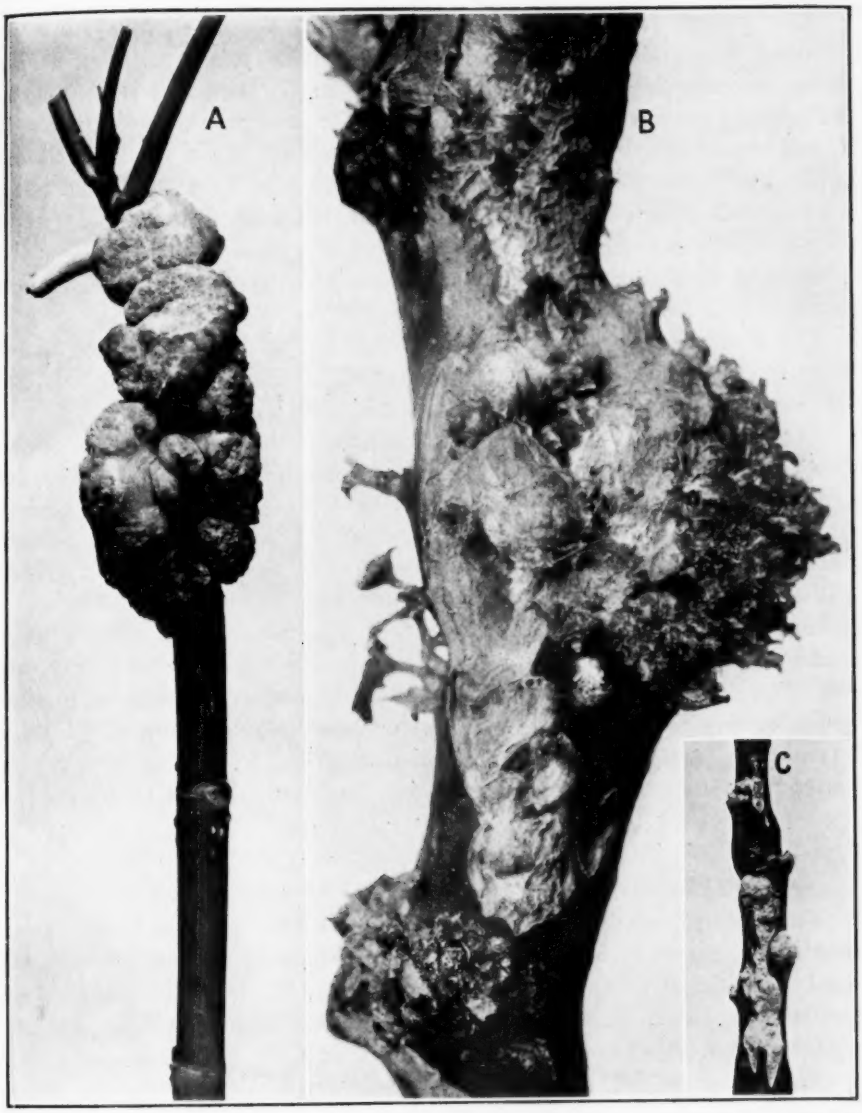

FIG. 4. A. Tumor on *Ricinus* (2/3 natural size) at end of 30 days for comparison with tumors shown in figure 3 at end of 4½ months. This tumor was produced with a sub-culture of col. 4 obtained from the tumors shown at the right in figure 3.

B. Inoculated slow-growing crowngall on *Begonia phyllomaniaca*. Right side of tumor full of tiny shoots. Juice pH 1.94. Time 84 days. Photo. Nov. 27, 1925. Twice natural size.

C. Slow-growing crowngalls on Vernon begonia. Juice pH 1.63. Time 100 days. 2/3 natural size. It may be compared with A.

(3) Tumors from the same. Only 2 cc. of a cloudy milky juice was obtained on grinding and extracting, and part of this came from normal tissues surrounding the tumors, and this naturally increased the acidity of the product beyond that which the pure tumor juice would have given. As it was impossible to test so small a quantity as 2 cc. in our calomel cell apparatus, it was diluted with distilled water up to 10 cc. and then tested with the following results: T. 25; mv. 512.0; pH 4.50; T.a. +45.

(4) For comparison with No. 3. The four stems immediately above, below and behind the tumors were crushed, extracted, centrifuged and tested. T. 25; mv. 396.0; pH 2.54; T.a. +88.

(5) For an exact comparison with No. 3, 2 cc. of No. 4 were now diluted to 10 cc. with the distilled water and tested. T. 25; mv. 420.9; pH 2.96; T.a. +80, appr. Dupl. T. 25; mv. 420; pH 2.94; T.a. +84, appr.

It is thus evident that the normal acidity of the begonia tissue was reduced locally by the growth of the tumor and this is in keeping with our experiments on other plants. For example the juice from soft Ricinus stems like those shown in figures 2 and 4 has a pH of 5.53 to 5.54 (several tests), whereas the tumor juice is more alkaline, ranging from pH 5.74 to 5.79 (several tests). To what extent the acidity of the begonia tissue was reduced it is impossible to determine from this experiment. We think to somewhere between pH 5 and pH 6. Cultures from the smaller (left hand) gall (Fig. 3) yielded nothing on the agar plates, nor anything in the original bouillon, in which the whole of it was crushed. Mixed juice of the other 3 tumors (a loop full) into bouillon yielded typical colonies on agar poured plates with subcultures, from one of which the tumor was reproduced on *Ricinus* (Fig. 4A).

ACIDITY OF OTHER PLANTS IMMUNE OR SEMI-IMMUNE TO CROWNGALL

For a long time we have known that olive shoots, onion shoots, garlic shoots, and shoots of various other plants, especially monocotyledons, can not be inoculated very successfully with crowngall (many unpublished experiments of Erwin F. Smith and Nellie A. Brown), but this is the first time potentiometer tests have been made.

Without going into details it may be said here that we found the following conditions in the juice of the plants known or supposed to be resistant to crowngall:

Pink skinned, small, very juicy onions (Allium cepa): pH 5.15. Total acidity, lost (a little too much alkali was added and the final reading was pH 8.54 (T.a. +50)). Dupl. pH 5.02. T.a. +43.

Green tops of brown skin onions: pH 4.33; T.a. +30. Dupl. pH 4.38; T.a. +32.

Green tops of silver skin onions: pH 4.45; T.a. + 30. Dupl. pH 4.50; T.a. + 34.

White dry garlic bulbs (Allium sativum) from Italy: pH 3.53. T.a. lost. Dupl. pH 3.46; T.a. + 165.

Elephant's-ear (Colocasia antiquorum) leaves: pH 5.33; T.a. + 10.

Eleven petioles from 6 small plants, pH 5.72; T.a. + 9. Dupl. pH 5.71; T.a. + 8.

Three inner petioles from 2 large plants, pH 5.65; T.a. + 8. Dupl. pH 5.61; T.a. + 8.

Young shoots of olive (Olea europea): pH 5.22; T.a. + 52; Dupl. pH 5.20; T.a. + 52; 2nd dupl. pH 5.28; T.a. + 52.

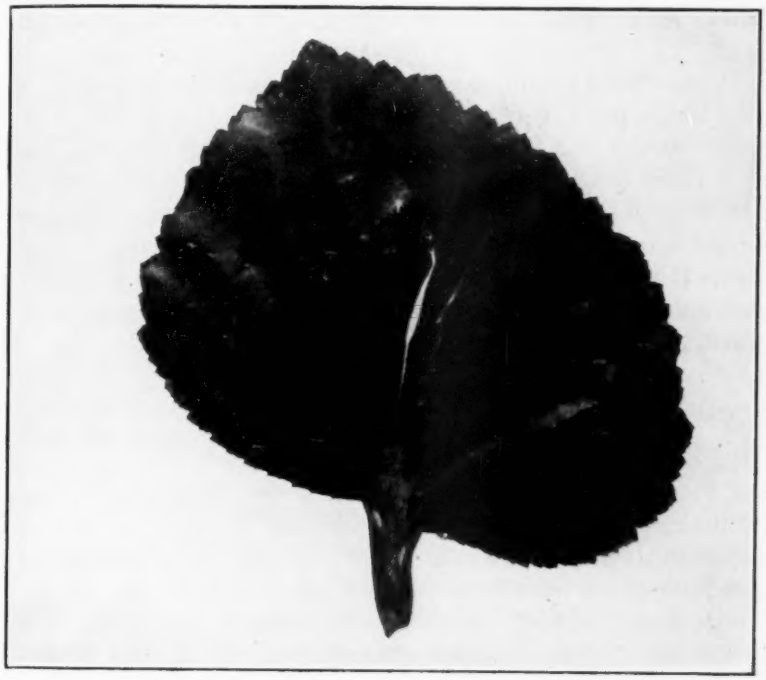

FIG. 5. Leaf of Vernon begonia (natural size). For stem see figure 4C.

Young petioles and midribs of banana (red variety): pH 5.02; T.a. + 14. Dupl. pH 5.04; T.a. + 14.

Young petioles and midribs of yellow fruited banana (Gros Michel): pH 4.74; T.a. + 12. Dupl. pH 4.70; T.a. + 12.

Juice of soft tops of sugar cane (var. Lahina), leaves excluded: pH 4.88; T.a. + 37. Dupl. pH 4.91; T.a. + 37. Another set: pH 4.82; T.a. + 60; dupl. pH 4.82; T.a. + 63.

The Wandering Jew (Tradescantia zebrina or Zebrina pendula) leaves: These leaves were broadly lanceolate, white-striped above and purple underneath. pH 5.47; T.a. between +8 and +10. Dupl. pH 5.44; T.a. +8.

Stems: pH 5.52; T.a. +21. Dupl. pH 5.52; T.a. +21.

Juice of young pineapple leaves (the smooth leaved variety commonly cultivated in the Panama Canal Zone): pH 4.72; T.a. between +38 and +40. Dupl. pH 4.73; T.a. +39.

Boston Market fern (Nephrolepis exaltata var. Bostoniensis): (32 fronds) pH 5.20; T.a. +38. Dupl. pH 5.22; T.a. +38.

Holly fern (Polystichum lonchitis, also called Aspidium): (25 fronds) pH 5.35; T.a. between +41 and +42. Dupl. pH 5.32; T.a. +42.

Rumex crispus (leaves): pH 3.53; T.a. +66. Dupl. pH 3.56; T.a. +66.

Rumex obtusifolius (40 leaves) pH 3.64; T.a. +68. Dupl. pH 3.67; T.a. +69.

Hothouse Oxalis (acaulescent, big-leaved, pink-flowered form). Several hundred leaves, pH 1.70; T.a. +224. Dupl. pH 1.72; T.a. +224.

Agave (young leaves): pH 4.30; T.a. +100. Dupl. pH 4.31; T.a. +102.

At the time the tests were made the H-ion ranges of all of these plants were believed to be too acid for the growth of the crowngall organism, and probably the tumor never does occur on them in nature, but we now think it possible to produce it on many of them by excessive doses of the organism. It is not unlikely that the preponderant kind of acid they contain has more to do with the resistance than the total acidity.

SUMMARY OF RESULTS

Begonia lucerna

(1) This is the most acid plant we have ever tested and the concentration of the acid is surprising; (2) the leaves are the most acid part; (3) the acidity of the stem decreases from top to base, *i.e.*, from young to old parts; (4) the juice of the leaves was twice as acid as lemon juice and almost or quite as acid as N/10 HCl;[3] (5) a very considerable part of the acid of this begonia is oxalic acid, but other acids are present; (6) the old nematode tumors were the least acid parts of the plant both in pH value and by electrometric titration; (7) inasmuch as pH 5.70 represents about the limit of growth of *Bact. tumefaciens* in bouillon media and in acid juices, the reason for its failure to grow in this begonia was assumed to be the acidity

[3] The juice of ripe lemons was found to be pH 2.20 (one test and two duplicates, in which we got precisely the same readings). The most astonishing discovery was the buffer content of the lemon juice, the total acidity, as determined by titration on the potentiometer down to pH 8.2 (phenolphthalein neutrality), being around +950 on Fuller's Scale, *i.e.*, it would take 950 c.c. of N/1 NaOH added to 1 liter of the juice to reach the pH 8.2 point in the titration curve.

of its tissues, since it grows readily in the juice of *Begonia lucerna* when its acidity is reduced to pH 6.56, and still better when it is reduced to pH 7.10; (8) *Heterodera radicicola* tolerates a more acid substratum than *Bact. tumefaciens*, and like the latter, when it is able to grow, changes the substratum to suit its needs, *i.e.*, makes it more alkaline when it is too acid; (9) exposure of the expressed juice of this begonia to the air for an hour or two made no appreciable change in its acidity (H-ion content); (10) there are two interesting indicators in this plant. The one in the stem juice is colorless between mv. 349 and mv. 461 (pH 1.77 to pH 3.71); pale blue becoming pure blue between mv. 505 and mv. 554 (pH 4.39 to pH 5.75); deep violet blue at mv. 622 (pH 6.32); and purple red changing to brown red between mv. 663 and mv. 911 (pH 7.01 to pH 11). The end term of the other indicator is brown green.

Other Begonias

What proved true of *B. lucerna* was found to be true of all the other begonias tested; they all have a strongly acid juice. All contain oxalic acid but the relative amounts are not known. All are rather resistant to crowngall inoculations, but this resistance may be overcome by mass action of the bacteria to some extent at least.

Other Plants

All the other plants known or believed to be immune to crowngall were found to have a juice considerably more acid than pH 5.70, the limit of crowngall toleration in bouillon, but all, with the exception of the *Oxalis*, were less acid than the begonias. The plants tested were *Oxalis*, onion, garlic, olive, pineapple, banana, sugar cane, elephant's-ear, *Rumex* (2 sp.), agave, and two ferns.

Assumptions as to pH

We started out with the assumption that the pH controlled tumor formation and that no tumors could be produced on plants having a greater acidity than pH 5.00, but the final experiments show that we have been able to produce crowngall tumors on three begonias having a very much more acid juice reaction than any the organism will tolerate in fluid cultures. The only conclusion we have come to is that mass action must control infection, *i.e.*, if you can once get a tumor started in a tissue, even in a very acid tissue, it will make its own milieu and continue to grow in spite of the inhibiting pH. This probably would be true even on *Begonia lucerna*.

Considering these results, it is premature to state that no crowngall tumors can be produced on the plants mentioned and supposed hitherto to be immune owing to their acid juice. Further experiments should be made, but it is still believed that crowngall tumors on such plants are non-existent

in a state of nature. Other factors than acidity of course may determine resistance in some of these plants.

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FUSARIUM ROT OF GLADIOLUS CORMS

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The two most important fungous diseases of gladiolus corms are those to which the names hard rot (2) and dry rot (3) have been given. These are to be found in practically all collections of any size, whereas a third disease, Fusarium rot (4), is sometimes present in sufficient amount to be given equal rank in importance.

Fusarium rot was observed as early as 1912. Although it received casual attention over a period of several years, no special investigation of it was undertaken by the writer until 1916. At about this time what seemed to be the same fungus was isolated from so many diseased corms sent to the Department of Plant Pathology at the New York State College of Agriculture that it was considered advisable to make a more intensive study of the disease and its cause.

Diseased corms have been received from a sufficiently large number of growers in New York to warrant the conclusion that Fusarium rot is of general occurrence in this state. Corms affected with the disease also have been received from Maryland, District of Columbia, New Jersey, Ohio, Indiana, Michigan, Wisconsin, Minnesota, California, and Ontario, Canada. Whether or not the plants were grown by the growers who sent the diseased material has not always been clear. However, with the general practice of growers buying relatively large quantities of corms from one another, it seems probable that diseased corms have been distributed throughout the United States and that the disease occurs in some degree in all areas suitable to the growth and parasitism of the fungus.

No instances of a high percentage of diseased corms in large collections, as is common in the case of both the hard rot and the dry rot, have come to the attention of the writer. In these latter diseases, especially hard rot, it is not uncommon to find over 90 per cent diseased corms in collections numbering several hundred thousands or more. However, three cases have been noted in which over 75 per cent of certain small lots of corms have been found to be affected with Fusarium rot. It is interesting to note that, whereas inoculation experiments indicate the absence of varietal differences

[1] The writer is indebted to the Boyce Thompson Institute for Plant Research for laboratory and greenhouse facilities placed at his disposal during part of the course of this investigation.

in susceptibility, yet in these three cases the disease was more or less restricted to certain varieties.

Although certain workers have given attention to gladiolus diseases, literature contains no reference to this particular disease other than that made by the writer in 1922 (4). It is probable, because of the relatively greater importance of hard rot, dry rot and neck rot or scab (1), that these diseases have received first attention and that one or more of them were involved in cases where the identity of the disease is left uncertain in published accounts.

SYMPTOMS OF THE DISEASE

At the time of harvesting the corms in the autumn, Fusarium rot lesions appear as small water-soaked spots. They are of a reddish-brown color and occur in most cases on the sides and lower halves of the corms (Pl. XXV). It is usually necessary to remove the husks (sheathing leaf bases) from the corms in order to see the lesions, although in some cases the husk is also diseased, as indicated by its discoloration and unusual brittleness.

The rot advances during storage, the rate increasing with higher temperatures and increased humidities. Lesions are usually irregularly circular in outline and range in size from one-fourth to one inch in diameter depending upon conditions of storage and time of year. The entire corm may become involved and reduced to a dry, brownish-black, worthless mummy due to the coalescence of two or more lesions.

Perhaps the most characteristic symptom of this disease is the existence of prominent, irregularly concentric markings or ridges in the lesions (Pl. XXV, C). Unfortunately, the absence of these markings is not absolute proof that the disease is not Fusarium rot; however, they occur more often than not, and the writer has never observed them in lesions produced in any other gladiolus disease. They have been observed consistently on diseased corms of the varieties Fire King, Princeps and Mrs. Francis King.

The lesions are generally somewhat sunken due to the rapid drying and shrinking of the tissues. The advancing margins are usually definite and slightly raised, with active lesions showing a narrow, watersoaked outer area, dark in color, which blends sharply into the healthy tissue. Diseased tissue is hard and in most cases not over 5 to 7 millimeters in thickness. As is true of the hard rot, dry rot and scab, the diseased tissue, especially of less active lesions, can be chipped out with the finger nail, leaving apparently healthy tissue beneath.

The color of the lesions, although generally reddish-brown, varies somewhat with the variety affected. On Mrs. Francis King, hazel[2] is the predominating color; and on Fire King the color range is from burnt sienna to bay, with older lesions almost black.

[2] Designations of colors are according to Ridgway (5).

Fusarium rot is primarily a disease of stored corms, but certain conditions of disease in the field relate directly to the rot which advances during storage. Missing plants in the row may be due to the fact that the corms planted were worthless mummies, their rotted condition being concealed by the apparently healthy husks. Dwarfed, stunted plants, which frequently fail to produce blossoms, result from planting corms so severely diseased that they fail to develop root systems or conducting tissues sufficient to support the developing offspring. Diseased corms commonly decay prematurely in the field, saprophytes, in many if not most cases, playing an important rôle in the rapid disintegration of the tissues.

In two instances the pathogene causing this disease has been isolated from the base of decaying stems, and in one instance from lesions on the roots. In other instances species of *Fusarium* which might have been this fungus have been isolated from stems and roots, but the identities of the fungi were not determined absolutely. It seems certain, however, that the pathogene causing the corm rot may also cause a decay of the stem.

When corms affected with the Fusarium rot are kept under very moist conditions, the lesions commonly become covered with a grayish, mold-like growth of the fungus (Pl. XXV, A). Corms in this condition have been received from growers. Only in relatively few cases, however, has the fungus present on the surface been proved to be the pathogene here described. Under such conditions, common saprophytes are to be expected.

ETIOLOGY

The Fusarium rot of gladiolus is caused by the fungus *Fusarium oxysporum* var. **gladioli** n. var.

SOURCE OF PURE CULTURE

The culture of *Fusarium* referred to in the following descriptions and experiments was isolated from a typically diseased corm of the variety Fire King on February 9, 1925. Tissue plantings were first made, and from a subsequent transfer a strain from a single spore was obtained.

Simultaneously with the studies on which these descriptions are based, cultures of the fungus isolated from other varieties and reisolated from artificially infected corms have been studied sufficiently to eliminate any doubt of the strain from Fire King being pathogenic and being the one most commonly isolated from diseased corms. It is not maintained, however, that other Fusaria may not attack the gladiolus and cause corm rots similar to the one herein described.

MORPHOLOGY

The morphological characters (Figs. 1 and 2) of the organism, as determined from cultures on various media, are as follows:

Microconidia numerous, dominantly 0-septate. Macroconidia gradually attenuated toward both ends, nearly cylindrical in the middle half of their length, somewhat pedicellate, and usually uniformly curved throughout;

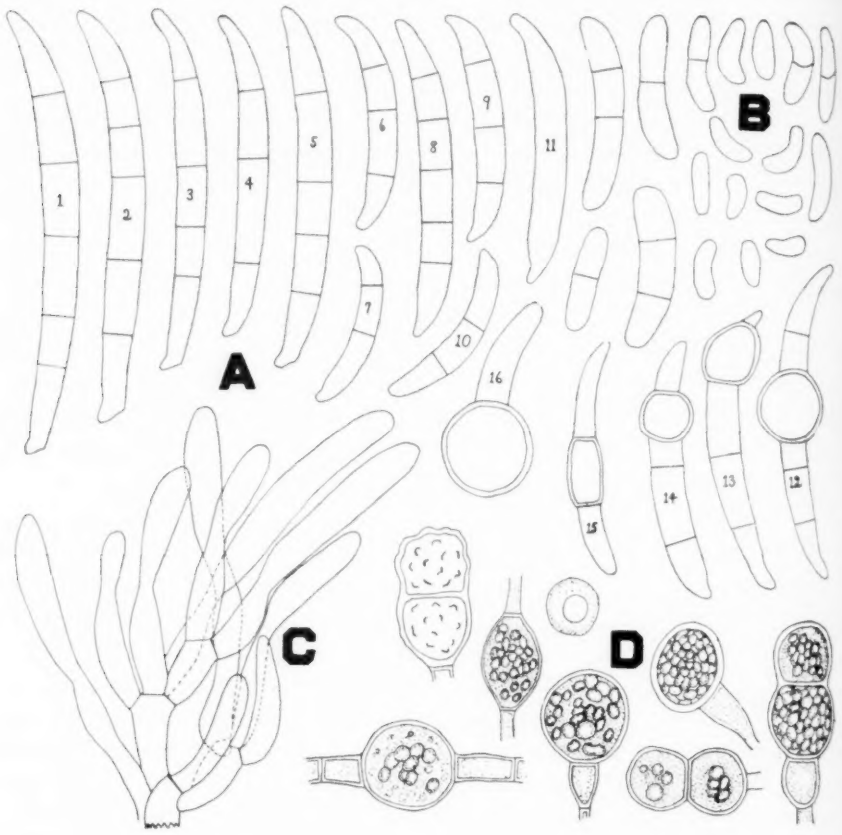

FIG. 1. Spores of *Fusarium oxysporum* var. *gladioli*. A. Macroconidia: 1, 10, from a medium sized sporodochium on hard potato agar after 25 days; 2, from a medium sized sporodochium on potato tuber plug after 39 days; 3, 4, 6, from a small sporodochium on tomato stem after 39 days; 5, 7, 8, from a large sporodochium on corn meal agar after 90 days; 9, 16, from a medium sized sporodochium on cornmeal agar after 38 days; 11, from a small sporodochium on tomato stem after 30 days; 12, 13, 14, 15, from a large sporodochium on tomato stem after 94 days. B, microconidia from culture on hard potato agar after 19 days. C, conidiophore from culture on tomato stem after 8 days. D, chlamydospores from culture on tomato stem after 94 days. Outlined with camera lucida, x 1000.

0-septate up to 98 per cent, 35 x 4.2 (30–42 x 3.7–4.6) μ; 1-septate up to 6 per cent, 20.5 x 3.2 (19–21 x 3.1–3.4) μ; 2-septate up to 10 per cent, 28 x 4.0 (27–30 x 3.9–4.1) μ; 3-septate, dominant, up to 92 per cent, 33.4 x 4.3 (25–41 x 3.5–4.8) μ; 4-septate up to 47 per cent, 39 x 4.4 (33–43 x 3.8–4.8) μ; 5-septate up to 8 per cent, 44 x 4.5 (42–46 x 4.3–4.6) μ; 7-septate, less than 1 per cent, 42 x 4.8 (40–44 x 4.8) μ. Chlamydospores mostly 0-septate, approximately globose and smooth-walled, intercalary or terminal, and commonly present in conidia; in conidia, 0-septate, 9 x 7 (6–14 x 5–10) μ; in mycelium, 0-septate, 11 x 10 (7–17 x 7–16) μ. Aerial mycelium present on most media, typically white and well developed, loosely-cottony, up to 5 mm. in height. Sporodochia up to 2 mm. in diameter, forming in from 7 to 14 days, typically salmon-buff in color. Bluish-black sclerotia present on potato tuber plug and commonly present on other media.

CULTURAL CHARACTERS

Fusarium oxysporum var. *gladioli* was grown on the following media: cornmeal agar, with and without 5 per cent dextrose, in petri dishes, spore dilution cultures; hard potato agar neutral, with and without 5 per cent dextrose, in petri dishes and test tubes; hard potato agar, made acid by the addition of one drop of 50 per cent lactic acid to 10 cubic centimeters of the medium, with and without 5 per cent dextrose, in petri dishes and test tubes; oatmeal agar, in petri dishes and test tubes; lima bean agar, in test tubes; rice, 2 grams in 6 cubic centimeters of water in test tubes; potato tuber cylinders, in test tubes, no water added; sweet clover, raspberry, tomato and potato stems in test tubes, each with 4 cubic centimeters of water added. The vegetable decoctions were prepared according to the formulae given by Sherbakoff (7, p. 105). All agar media contained 3 per cent agar. All media were autoclaved for 45 minutes at 10 pounds pressure, except rice, which was steamed for one hour on three successive days. With the exception of cultures on cornmeal agar, which were spore dilutions, all were initiated by transferring bits of medium containing mycelium from other cultures. The cultures were kept in diffuse daylight at room temperature (approximately 22° C.).

No marked variations or unusual types of growth occurred on any of the media. It was not difficult to maintain the cultures in the state of *Hochkultur* over a period sufficiently long to complete the taxonomic study. The intensity of color production was increased by the addition of lactic acid and of dextrose. The growth rate, as measured by the diameter of the thalli in petri dishes, was slightly retarded by the addition of the lactic acid and slightly increased by the addition of 5 per cent dextrose. Aerial mycelium was present and typically well developed in all cultures with the

exception of those in which the fungus was growing on cornmeal agar. It was 3 to 5 mm. in height, of a loose-cottony appearance becoming slightly more woolly and appressed with age, and dominantly white. In cultures where the aerial mycelium was colored, as for example, hard potato agar with 5 per cent dextrose, the color varied from pinkish vinaceous through dark vinaceous to ox-blood red, representing a diffused color of the stroma. On rice, when 60 days old, the aerial mycelium was dominantly white, ranging to pinkish vinaceous. Sporodochia formation was retarded or entirely prevented by the addition of lactic acid or 5 per cent dextrose. Mature sporodochia were present in cultures on cornmeal agar with 1.5 per cent dextrose in 16 days, and abundant on oat agar in 24 days. They were formed readily and in large numbers on stems of raspberry, tomato, sweet clover, and potato, being mature in about 14 days. They were typically salmon-buff in color.

Only in those cultures in which the fungus was growing in decoctions to which dextrose or lactic acid, or both, had been added, and in cultures on rice and potato tuber plugs, did the substratum become colored. A deep purplish vinaceous color developed in from 5 to 7 days. In about 15 days, in petri dish cultures on potato agar, the color of the substratum became almost black in the center of the dish, grading to the outer edge through bordeaux, dark vinaceous purple and ox-blood red. On rice, when 60 days old, the substratum was dominantly pinkish vinaceous, grading through dark vinaceous to bluish-black deeper in the medium.

In determining the size of the conidia, at least 50 and in some instances 100 spores were measured. The measurements on the various media are as follows:

On cornmeal agar, culture 16 days old; conidia taken from a small sporodochium:
0-septate, 98 per cent, 41.2 x 4.7 (30.0–50.4 x 4.2–6.0) μ.
3-septate, 2 per cent, 42 x 4.8 μ.

On tomato stems, culture 18 days old; conidia taken from a large sporodochium:
0-septate, 92 per cent, 31.5 x 4.6 (25.2–45.6 x 3.6–5.4) μ.
3-septate, 8 per cent, 29.7 x 4.8 (25.2–37.2 x 4.8) μ.

On tomato stems, culture 19 days old; conidia from a large sporodochium:
0-septate, 30 per cent, 35.1 x 4.1 (27.6–49.2 x 3.6–4.8) μ.
2-septate, 4 per cent, 33.6 x 3.6 (31.2–36.0 x 3.6) μ.
3-septate, 52 per cent, 35.1 x 4.0 (27.0–48.0 x 3.0–4.8) μ.
4-septate, 14 per cent, 43.0 x 4.1 (38.4–50.4 x 3.6–4.8) μ.

On potato stem, culture 21 days old; conidia from a medium sized sporodochium:
0-septate, 34 per cent, 29.5 x 3.6 (21.6–36.0 x 3.0–4.2) μ.
1-septate, 2 per cent, 19.2 x 3.0 μ.
2-septate, 10 per cent, 26.2 x 3.7 (19.2–31.2 x 3.6–3.9) μ.
3-septate, 54 per cent, 29.8 x 3.9 (24.0–36.0 x 3.0–4.8) μ.

On potato tuber plug, culture 21 days old; conidia taken from a small sporodochium:
0-septate, 30 per cent, 32.8 x 4.2 (28.8–45.6 x 3.6–4.8) μ.

3 septate, 44 per cent, 30.4 x 4.3 (20.4–38.4 x 3.0–4.8) μ.
4-septate, 24 per cent, 35.0 x 4.5 (30.0–39.6 x 3.6–4.8) μ.
5-septate, 2 per cent, 37.2 x 4.2 μ.

On sweet clover stems, culture 22 days old; conidia taken from a small sporodochium:

0-septate, 7 per cent, 27.8 x 4.0 (20.0–36.0 x 3.3–4.2) μ.
3-septate, 83 per cent, 32.6 x 4.3 (20.0–42.0 x 3.6–4.8) μ.
4-septate, 10 per cent, 37.4 x 4.2 (33.6–40.8 x 3.6–4.8) μ.

On locust stems, culture 22 days old; conidia taken from a small sporodochium:

1-septate, 4 per cent, 24.0 x 3.3 (20.4–27.6 x 3.0–3.6) μ.
2-septate, 2 per cent, 31.2 x 3.6 μ.
3-septate, 86 per cent, 31.3–4.1 (21.6–39.6 x 3.0–4.8) μ.
4-septate, 8 per cent, 34.8 x 4.0 (32.4–37.2 x 3.6–4.5) μ.

On hard oat agar, culture 23 days old; conidia from a large sporodochium:

3-septate, 54 per cent, 34.7 x 4.4 (26.4–46.2 x 3.6–4.8) μ.
4-septate, 44 per cent, 41.5 x 4.4 (34.8–48.0 x 4.2–4.8) μ.
5-septate, 2 per cent, 45.6 x 4.2 μ.

On hard oat agar, culture 25 days old; conidia taken from a medium sized sporodochium:

0-septate, 2 per cent, 39.6 x 3.6 μ.
2-septate, 2 per cent, 32.4 x 4.8 μ.
3-septate, 48 per cent, 35.3 x 4.5 (24.0–45.6 x 3.6–4.8) μ.
4-septate, 40 per cent, 44.2 x 4.5 (36.0–56.4 x 4.2–4.8) μ.
5-septate, 8 per cent, 47.1 x 4.4 (44.4–49.2 x 4.2–4.8) μ.

On hard oat agar, culture 25 days old;

(a) Conidia taken from a medium sized sporodochium:

3-septate, 71 per cent, 34.8 x 4.3 (25.2–44.4 x 3.0–4.8) μ.
4-septate, 28 per cent, 39.1 x 4.5 (33.6–48.0 x 3.6–4.8) μ.
5-septate, 1 per cent, 55.8 x 4.8 μ.

(b) Chlamydospores in mycelium:

0-septate, 100 per cent, 10.6 x 11.0 (6.0–14.4 x 7.2–15.6) μ.

On hard potato agar, culture 36 days old;

(a) Conidia taken from a medium sized sporodochium:

1-septate, 6 per cent, 20.8 x 3.2 (19.2–21.6 x 3.0–3.6) μ.
2-septate, 2 per cent, 25.2 x 3.6 μ.
3-septate, 62 per cent, 33.8 x 4.1 (27.6–39.6 x 3.6–4.8) μ.
4-septate, 30 per cent, 38.4 x 4.2 (36.0–45.6 x 3.9–4.8) μ.

(b) Chlamydospores in conidia:

0-septate, 98 per cent, 8.7 x 6.8 (6.0–10.8 x 4.8–8.4) μ.
1-septate, 2 per cent, 9.6 x 6.0 μ.

On cornmeal agar, culture 36 days old; conidia taken from a large sporodochium:

2-septate, 1 per cent, 19.2–3.6 μ.
3-septate, 55 per cent, 33.2–4.2 (21.6–44.4 x 3.0–4.8) μ.
4-septate, 38 per cent, 38.1 x 4.4 (32.4–48.0 x 3.6–4.8) μ.
5-septate, 6 per cent, 40.8 x 4.7 (38.4–45.6 x 4.2–4.8) μ.

On hard oat agar, culture 38 days old; conidia taken from a large sporodochium:

3-septate, 74 per cent, 32.8 x 4.3 (26.4–39.6 x 3.6–4.8) μ.
4-septate, 18 per cent, 39.6 x 4.1 (33.6–44.4 x 3.6–4.2) μ.
5-septate, 8 per cent, 47.1 x 3.7 (42.0–52.8 x 3.6–4.2) μ.

On cornmeal agar, culture 38 days old;

(a) Conidia taken from a medium sized sporodochium:

2-septate, 2 per cent, 27.6–4.2 μ.
3-septate, 58 per cent, 34.1 x 4.2 (24.0–38.4 x 3.3–4.8) μ.
4-septate, 32 per cent, 38.3 x 4.5 (32.4–48.0 x 3.6–4.8) μ.
5-septate, 8 per cent, 41.1 x 4.7 (38.4–43.2 x 4.5–4.8) μ.

(b) Chlamydospores in conidia:

0-septate, 100 per cent, 9.7 x 8.9 (4.8–15.6 x 4.8–14.4) μ.

On potato tuber plug, culture 39 days old; conidia taken from a medium sized sporodochium:

0-septate, 2 per cent, 38.4 x 4.8 μ.
2-septate, 6 per cent, 21.6 x 3.8 (20.4–22.8 x 3.6–4.2) μ.
3-septate, 78 per cent, 29.3 x 4.3 (20.4–38.4 x 3.6–4.8) μ.
4-septate, 14 per cent, 32.7 x 4.5 (24.0–39.6 x 4.2–4.8) μ.

On tomato stem, culture 39 days old;

(a) Conidia from a small sporodochium:

0-septate, 1 per cent, 38.4 x 4.2 μ.
1-septate, 1 per cent, 15.6 x 3.0 μ.
2-septate, 3 per cent, 32.8 x 4.1 (27.0–38.4 x 3.6–4.5) μ.
3-septate, 83 per cent, 36.8 x 4.4 (19.2–44.4 x 3.6–4.8) μ.
4-septate, 11 per cent, 41.1 x 4.4 (34.8–51.6 x 4.2–4.8) μ.
5-septate, 1 per cent, 40.8 x 4.8 μ.

(b) Chlamydospores in conidia:

0-septate, 100 per cent, 8.3 x 6.6 (6.0–11.4 x 4.8–7.8) μ.

On cornmeal agar, culture 90 days old; conidia taken from a medium sized sporodochium:

1-septate, 1 per cent, 22.8 x 3.6 μ.
2-septate, 2 per cent, 21.0 x 3.9 (19.2–22.8 x 3.6–4.2) μ.
3-septate, 40 per cent, 32.9 x 4.5 (24.0–39.6 x 3.6–5.4) μ.
4-septate, 47 per cent, 36.7 x 4.7 (30.0–50.4 x 4.2–5.4) μ.
5-septate, 8 per cent, 40.8 x 4.7 (34.8–44.4 x 4.2–4.8) μ.
7-septate, 2 per cent, 42.0 x 4.8 (39.6–44.4 x 4.8) μ.

On hard oat agar, culture 92 days old; chlamydospores in conidia:

0-septate, 100 per cent, 9.6 x 6.6 (6.0–16.8 x 4.5–9.6) μ.

On tomato stem, culture 94 days old; chlamydospores in mycelium:

0-septate, 92 per cent, 12.0 x 10.7 (7.8–19.2 x 7.2–15.6) μ.
1-septate, 8 per cent, 19.2 x 12.6 μ.

On locust stems, culture 105 days old; conidia taken from a medium sized sporodochium:

0-septate, 4 per cent, 33.6 x 4.1 (30.0–37.2 x 3.6–4.5) μ.
3-septate, 92 per cent, 32.3 x 3.8 (21.5–44.5 x 3.0–4.8) μ.
4-septate, 4 per cent, 41.2 x 4.2 μ.

Average of the above measurements:[3]

Conidia: 0-septate, 30 per cent, 34.8 x 4.2 (30.0–41.6 x 3.7–4.6) μ.
1-septate, 3 per cent, 20.5 x 3.2 (19.4–21.4 x 3.1–3.4) μ.
2-septate, 3 per cent, 28.4 x 4.0 (26.8–29.9 x 3.9–4.1) μ.
3-septate, 58 per cent, 33.4 x 4.3 (25.0–41.0 x 3.5–4.8) μ.
4-septate, 26 per cent, 38.6 x 4.4 (33.0–42.7 x 3.8–4.8) μ.

[3] Percentages calculated on the basis of cultures in which the indicated conidia were found—not on total number of cultures studied.

5-septate, 5 per cent, 44.0 x 4.5 (41.9–46.1 x 4.3–4.6) μ.
7-septate, less than 1 per cent, 42.0 x 4.8 (39.6–44.4 x 4.8) μ.

Chlamydospores:
(a) in conidia, 0-septate, 99 per cent, 9.1 x 7.2 (5.7–13.6 x 4.7–10.5) μ.
(b) in mycelium, 0-septate, 96 per cent, 11.3 x 10.9 (6.9–16.8 x 7.2–15.6) μ.

From a comparison with descriptions by Wollenweber (9), Sherbakoff (7) and Wollenweber *et al.* (10), it is evident that this fungus (Figs. 1 and 2) falls in the section Elegans of the genus *Fusarium*, and within that group lies so close to *Fusarium oxysporum* Schlecht. emend. Wr. (6, 8) as to be considered a variety of this well-known species. It differs from *F. oxysporum* in having slightly longer macroconidia, a higher percentage of 4-septate spores, somewhat weaker development of the "foot" (Figs. 1 and 2), and especially in its parasitism (see under pathogenicity, below). For these reasons it would seem that the gladiolus fungus should be made at least a new variety, the alternative being to make it a new species.[4]

PATHOGENICITY

The ability of *Fusarium oxysporum* var. *gladioli* to infect the gladiolus and produce lesions on corms typical of those from which the fungus has been isolated many times has been established through numerous experiments both in the field and under greenhouse conditions. Infection takes place when the fungus is added to the soil before the corms are planted, when the corms are dipped in suspensions of spores from a pure culture and either planted in soil or else held in moist chambers in the laboratory, and when inoculations are made with the pathogene into newly developing corms from healthy corms planted in clean soil.

On March 28, 1916, 12 plants, variety Meadowvale, growing in the greenhouse bench were inoculated when they were from 8 to 10 inches in height. The soil was removed from about the corms and a bit of potato agar carrying the fungus was pressed against each of the uninjured corms. The inoculum was then covered with cotton saturated with water, and the soil replaced. When an examination was made on April 12, at which time the experiment was discontinued, it was found that lesions typical of Fusarium rot were present on 8 of the 12 corms. Check plants remained healthy. The fungus was reisolated from these lesions and its identity determined.

In the summer of 1921 infection was obtained by inoculating corms of the varieties Princeps, Augusta, Peace, Mrs. Francis King, America, Fire King, Meadowvale, and three unknown varieties, as well as Primulinus

[4] Assistance in the determination of the fungus was given by Dr. C. D. Sherbakoff, who concurred with the writer in his decision that the fungus should be made a variety of *F. oxysporum* Schlecht. emend. Wr.

Hybrids. These were growing in an experimental garden in soil in which no gladioli had ever been grown. The parent corms were planted June 1, and the corms developing from them were inoculated during August when they were approximately one inch in diameter. Of the 15 corms of each

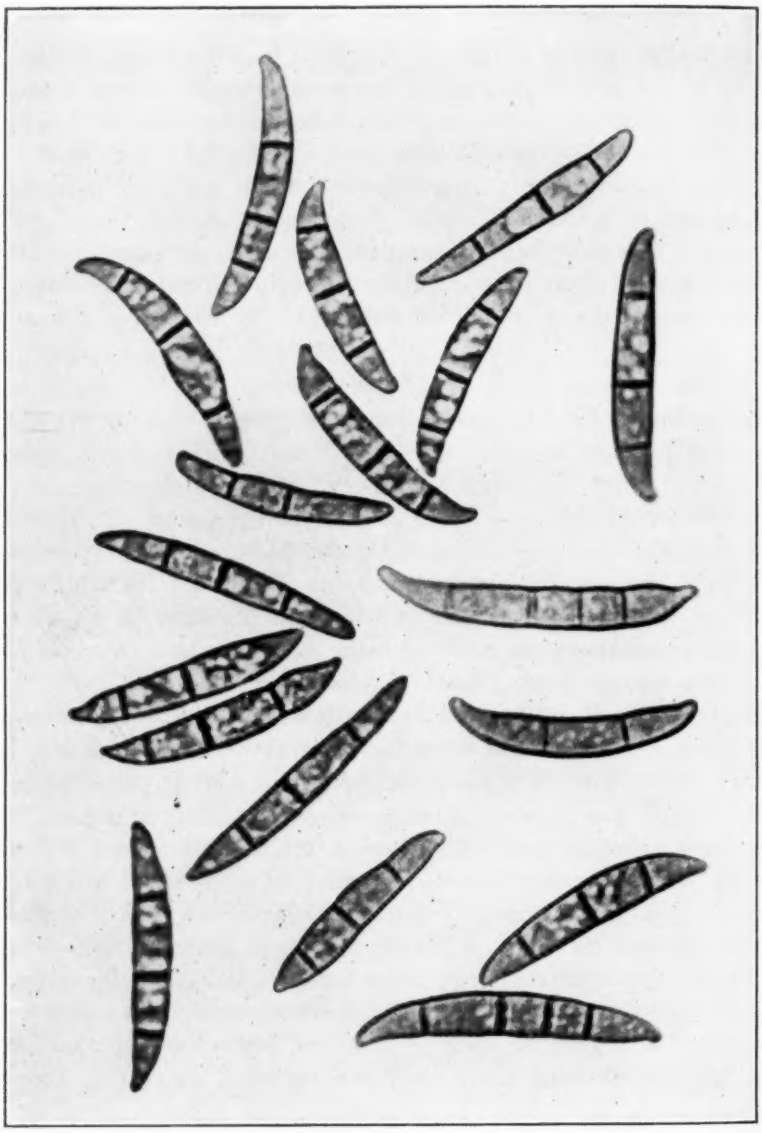

FIG. 2. Macroconidia of *Fusarium oxysporum* var. *gladioli*, from medium sized sporodochium on sweet clover stem after 63 days. Photomicrograph, x 800.

variety inoculated, 5 were injured by making several cuts through the sheathing leaf bases into the body of the corms, and the remainder uninjured. The inoculum was taken from a two months' old culture of the fungus on sterilized wheat kernels. The method involved pulling away the soil from the base of the plant and pressing about six kernels of wheat carrying the fungus against the injured or uninjured surface. The inoculum was then covered with cotton saturated with water and the soil replaced. Five plants of each lot were given similar treatment with the exception that the fungus was omitted. No evidences of disease, as indicated on plant parts above ground, developed prior to harvest in October, and the corms were not examined until the following January. Infection was obtained on all varieties, and the checks were found to be sound. The percentages of infection were 90 and 68, respectively, of those injured and uninjured.

That infection may be obtained under artificial conditions was proved by the following experiment. Fifty large corms of the variety Mrs. Francis King were selected as being sound, no lesions being evident from a critical examination following the removal of the husks. They were then placed in 50 per cent alcohol for 3 minutes, in 1–1,000 mercuric chloride for 15 minutes, given five washings in distilled water, and then placed on moistened filter paper in moist chambers, 240 by 80 mm., which had been sterilized previously. Two kinds of inoculum were used. The first consisted of bits of medium bearing the fungus, taken from a 9-days old culture on potato agar; the second, a suspension of spores from sporodochia produced on tomato stems. In the first case the method consisted in pressing the inoculum against the uninjured surface of the corms. In using the second type of inoculum, somewhat less than one cubic centimeter of the spore suspension was dropped into the depressions occurring naturally on the upper surfaces of the corms. Each inoculated lot consisted of 20 corms, and 10 corms were carried as checks. The moist chambers were kept in a constant temperature chamber at 25° C., and water was added as needed to keep the filter papers saturated.

The inoculations were made on April 30, 1921. On June 10, lesions measuring from one-half to one inch in diameter were present on all the inoculated corms. By July 15, the rot involved from one-half to the entire surface of the corms, and a mold-like growth of the fungus covered the lesions. This growth was powdery in appearance, due to the large numbers of spores, and numerous sporodochia also were present. The checks remained free from decay.

Several other experiments similar to the above have been conducted at various times and have always yielded a high percentage of infection. Some of the earlier experiments gave results indicating that varieties with dark

colored corms were more resistant than those with white and light yellow corms, but later experiments failed to substantiate this possibility.

Infection has been secured by inoculating uninjured stems at a distance of 8 to 12 centimeters above the surface of the soil. In all cases the inoculum was covered with cotton, which was kept moist by adding water from time to time during the first week following inoculation. In about three weeks the rot had advanced down the stem to below the soil line and had progressed above the point of inoculation about 5 centimeters. The foliage became yellow and died prematurely.

No infection with *F. oxysporum* var. *gladioli* has been obtained in several trials on living stems of tomato, pepper, egg-plant and potato. In one experiment, negative results were obtained on potato plants under conditions which yielded infection with a culture of *F. oxysporum* obtained from Dr. C. D. Sherbakoff. The inoculations were made by introducing the fungi growing on wheat kernels into longitudinal slits in potato stems at about the surface of the soil. The plants were growing in the greenhouse and were from 6 to 8 inches in height when inoculated. The stems at the point of inoculation were wrapped with moistened cotton which was not removed throughout the duration of the experiment. Twenty stems were inoculated with each of the two fungi. The check consisted of the same number of stems given similar treatment, except that no fungous growth was introduced into the stems along with the wheat kernels.

The inoculations were made on August 15, 1925. On September 15 it was found that 15 of the 20 stems inoculated with *F. oxysporum* were infected, as evidenced by the discoloration of the vascular tissues for a distance of about 2.5 centimeters below and 6.5 centimeters above the point of inoculation. No pronounced wilting was observed. Tissue plantings were made from these stems and the fungus obtained in culture. In the case of the gladiolus fungus, a very slight discoloration, less than 1.5 centimeter in total extent, occurred at the point of inoculation, but there were no indications that the fungus was pathogenic to potatoes. It would seem, then, that the parasitism of *Fusarium oxysporum* var. *gladioli* is not identical with that of *Fusarium oxysporum* from potatoes.

TEMPERATURE RELATIONS

Cultures of *F. oxysporum* var. *gladioli* were established by transferring bits of a solid medium carrying mycelium to the center of petri dishes of equal size containing equivalent amounts of hard potato agar with 1 per cent dextrose. The medium was tested electrometrically and found to have a pH value of 7. After incubating all of the cultures for 24 hours at 20° C., they were divided into lots of four dishes each and each lot held for 10

hours at the particular temperature at which it was to be kept throughout the experiment. This permitted the fungus to establish itself in the medium in the petri dish and eliminated errors that might have arisen from failure to allow sufficient time for temperature adjustments before making the initial measurements. The diameters of the thalli were then recorded and the rate and amount of subsequent growth determined by measuring the increases over the original diameters. Four cultures were placed in each of a series of temperature chambers with the following temperatures: 0°, 5°,

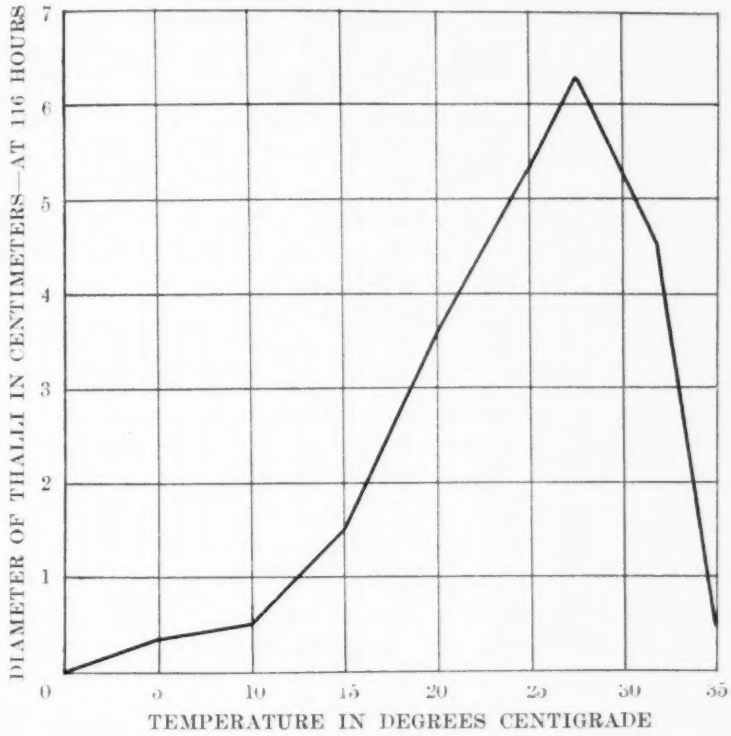

FIG. 3. Relation of temperature to growth of *Fusarium oxysporum* var. *gladioli* on hard potato agar with 1 per cent dextrose in petri dishes.

10°, 15°, 20°, 25°, 27.5°, 30°, 32° and 35° C. The diameters of the thalli at 116 hours were taken as a criterion of comparison, the results being represented graphically in figure 3. There is a gradual increase in rate of growth up to 25° with optimum near 27.5°, and a rapid drop beyond 30° C.

CONTROL

No experiments in the control of this particular disease have been attempted. From what is known regarding the nature of the disease, its

cause and the life history of the pathogene, it would seem that the problem of control would not differ greatly from that of the hard rot and dry rot diseases. For the latter two, it has been found that sorting, with the elimination of diseased corms, combined with a four or five years' rotation and proper handling of the corms at harvest and during storage are usually sufficient to keep the losses at a minimum. To be properly handled, the corms should be dug when they can be dried in the open for a day or two before being placed in storage. As little soil as possible, either adhering to or accompanying the corms, should be taken indoors. The old roots and adhering soil should be removed early during the storage period.

Throughout the first month the storage house should be given maximum ventilation to carry off the moisture and to facilitate drying. As early as possible in sections with cold winters, as in New York State, the temperature should be dropped to about 35° F., where it should be held throughout the winter.

Besides maintaining a low temperature, any practices which lower the humidity of the air about the corms is to be recommended. In instances where their use is feasible, trays with slatted rather than solid bottoms are preferred, and these are best stacked in racks which will permit the movement of air on all sides of them. The corms should not be piled deeper than 4 inches in the trays, and where possible should be stirred several times during the winter. Sorting, with the elimination of diseased corms, should be done late in the spring, since at this time the advanced stage of decay makes it easier to pick out those that are affected.

SUMMARY

Fusarium rot is an important disease of gladiolus corms in the United States. The disease probably occurs to some extent in all collections of gladioli of any considerable size. A description of the disease is given. Corms become infected in the field, and the rot advances during storage. Diseased corms in storage may be reduced to worthless mummies under favorable conditions for the disease, and the planting of infected corms frequently results in the death of plants in the field during mid-season.

The pathogene, *Fusarium oxysporum* var. *gladioli* n. var., belongs to the section Elegans, differing from *F. oxysporum* Schlecht. emend. Wr. in certain morphological characters and in its parasitism. Its morphological and cultural characters are recorded.

On potato agar, the fungus grows over a range of from about 5° to 35° C. Optimum growth takes place at about 27.5° C.

Infection was readily obtained on uninjured corms in the laboratory and on those growing in the greenhouse and experimental gardens.

Although no experiments on control were attempted, it is believed that sorting, with the elimination of diseased corms, together with crop rotation and the proper handling of corms at harvest and during storage, will greatly reduce losses from the disease.

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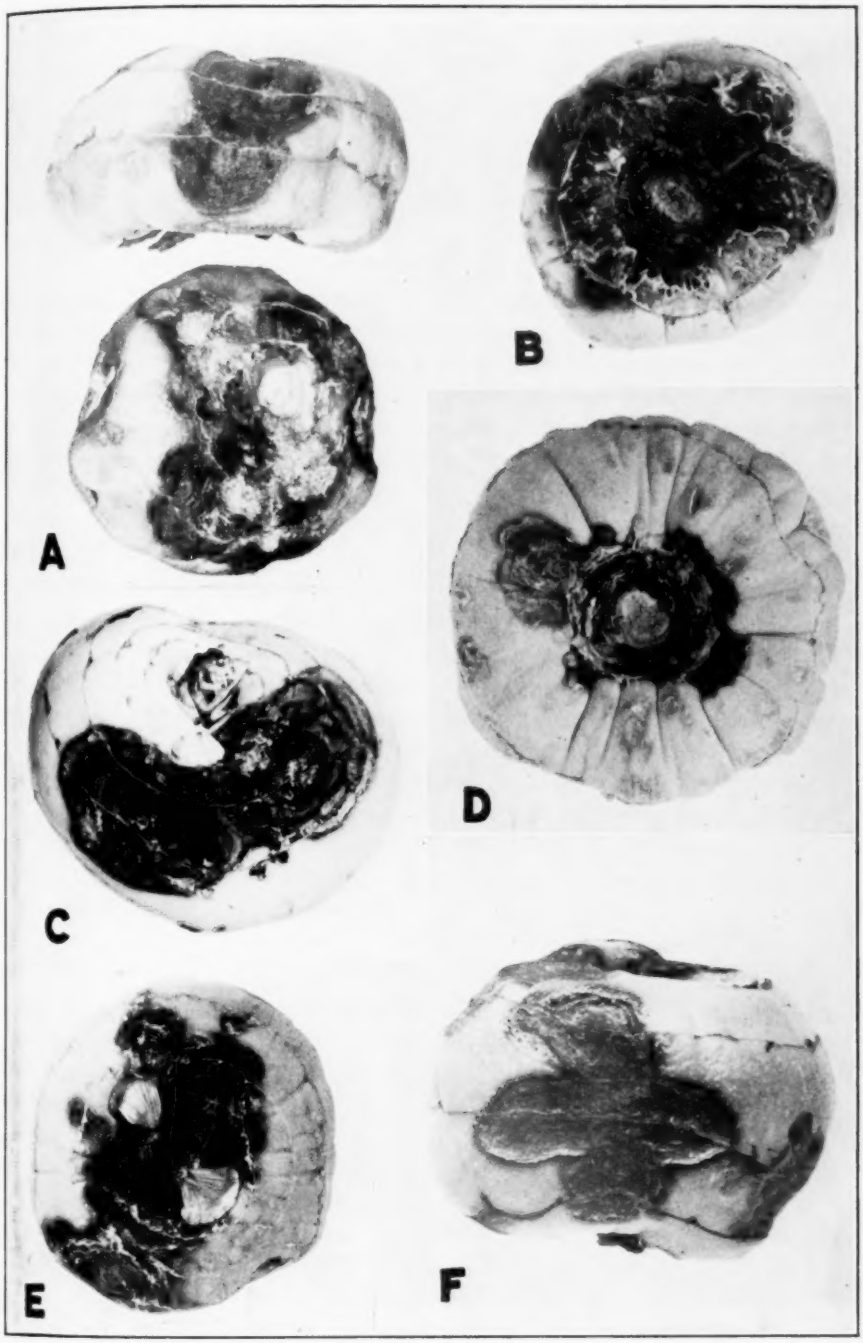
EXPLANATION OF PLATE XXV

Corms affected with *Fusarium oxysporum* var. *gladioli*. A. Variety Princeps, natural infection. B, E. Variety Fire King, natural infection. C. Variety Mrs. Francis King inoculated with pure culture of fungus isolated from Fire King and kept in moist chamber at 24° C. Photographed after 30 days. D. Variety America, natural infection. F. Unknown variety inoculated with pure culture of fungus isolated from Princeps and kept in moist chamber at 24° C. Photographed after 28 days.

All natural size with the exception of F which is x 1⅓.


A
B
C
D
E
F

SOME FACTORS AFFECTING THE PATHOGENICITY OF ACTINOMYCES SCABIES[1]

G. B. SANFORD[2]

INTRODUCTION

Common scab of potato, *Solanum tuberosum*, caused by *Actinomyces scabies* (Thaxter) Güssow, is prevalent and destructive. All attempts effectively to control the disease have failed. The potato scab pathogene is normally a soil saprophyte, and its activities are therefore influenced by other soil microorganisms. There may be metabiotic or antibiotic relations between these organisms, and these relations, as well as the soil flora, may be altered when one or more of the physical or chemical factors of the soil are changed, but the degree of this modification and its effect on scab development is not well understood.

A review of the work done for the control of potato scab previous to 1914 has been given by Lutman and Cunningham (9). Drechsler (3) has studied the morphology of *Actinomyces*, and Waksman (17) the physiology. Others have studied various methods of reducing scab. In addition to tuber disinfection, the principal preventive measure used has been to attempt to increase the acidity of the soil. This appears to have serious economic and other limitations on many potato soils, especially those having a high initial pH value and which are highly buffered. The plowing down of green crops, particularly rye, often is recommended as a control measure for potato scab, as the decomposition of organic matter thus put into the soil is assumed to increase soil acidity.

OBJECTS OF THE INVESTIGATION

The objects of this investigation were: to study the effect of temperature, moisture, oxygen supply, soil reaction, and stimulatory substances on the germination, growth and pathogenicity of *A. scabies*; and to throw additional light on the nature of the control said to be obtained by plowing under a green crop of rye.

[1] Published with the approval of the Director as Paper No. 618 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Grateful acknowledgment is made to Professor E. C. Stakman for his assistance and helpful criticism during this study.

METHODS

A synthetic dextrose-nitrate substrate was used for all studies, except where otherwise stated, the composition being: K_2HPO_4, 1 gm; $MgSO_4$, 0.5 gm; KCl, 0.5 gm; $FeSO_4$, trace; $NaNO_3$, 2 gm; dextrose 30 gm; agar 18 gm; distilled water 1000 cc. Agar slants in loosely plugged test tubes were found preferable to petri dish cultures. It was found advisable to add the spores[3] in suspension to the surface of the solid medium. Other methods will be described when necessary. A strain of *A. scabies* of tested pathogenicity was labelled "B" and used in all studies, except where otherwise stated.

EXPERIMENTAL RESULTS

Temperature Relations

The effect of temperature on spore germination has received attention by Shapovalov (15) and others, but a study of the influence of temperature on the pathogenicity of the strain of *A. scabies* used in these experiments seemed advisable.

Effect on growth and pathogenicity. Three strains of *A. scabies* (A, B and C) were used. Strains B and C were obtanied from S. A. Waksman, Rutgers College, N. J., and strain A was isolated by the writer and tested for pathogenicity. Duplicate cultures on nutrient agar slants were kept at the following temperatures: 7–8°, 10°, 13°, 16°, 19°, 22°, 24°, 26–27°, 29–30°, 32°, 35°, 37–38° C., and the growth recorded at 18-, 36-, 60-, 80- and 160-hour intervals. This experiment was repeated three times. All three strains reacted alike. The data for strain B are given in table 1.

TABLE 1.—*The effect of temperature on the growth of Actinomyces scabies, strain B*

Time in hours	Temperature in degrees Centigrade											
	7–8	10	13	16	19	22	24	26–27	29–30	32	35	37–38
	Rate of growth[a]											
18	0	0	0	0	0	1	2	4	5	4	2	0
36	0	0	0	0	1	3	4	4	6	5	3	0
60	0	0	0.5	1	4	5	6	7	8	6	3	1
80	0	trace	2	3	6	7	10[b]	12[c]	10	6	2	2
160	0	5	8	9	10	11	12[c]	12[c]	10	5	2	1

[a] Estimated value given to the relative progress of cultures after germination.

[b] Darkening.

[c] Dark.

[3] "Spores" is used in the widest sense because both conidia and mycelial segments function as reproductive structures.

It is evident from results that the range of temperature for growth increases with the time, and that germination and maturity take place sooner at the higher temperatures, with a corresponding decrease in vegetative growth. For instance, after 18 hours growth occurs between 22° and 35° C., while after 160 hours the range of temperature for growth extends from 10° to 38° C. Although good growth and maturity are attained at the lower temperatures in a longer time, the optimum point for greatest vegetative development and earliest maturity is 26–27° C. These results agree reasonably well with those of Lutman and Cunningham (9) who reported the optimum temperature to be 25° C.

Jones, McKinney and Fellows (6) stated that scab developed best at about 22° C., and that the amount decreased toward 11° and 30.5° C., where scab became negligible, as did also the yield of the tubers. They also suggested that the immediate effect of high temperature is more closely correlated with tuber development than with the growth of the pathogene. Scant growth of individual tubers, however, would not always explain the decrease of scab, because large scab pustules frequently form both on small and large tubers grown under field conditions. High temperatures may retard or prevent the growth of the scab pustule, subsequent to infection, but our knowledge of the process of tuber infection and scab development is insufficient to permit of conclusions.

The writer (14) obtained a maximum amount of scab in soils held at constant moisture content and temperatures of 14°, 17°, and 25° C.; and there was every indication that much scab would develop at at least two degrees lower or higher than 14° and 23° respectively. These results, with those of the present study, indicate that the growth of the pathogene in pure cultures, within the range of temperatures tested, is fairly well correlated with the amount of scab which develops. It might be concluded that temperatures lower than 12° C. will largely prevent scab development under field conditions and that periodic fluctuations between this point and 25° C. would not greatly affect the amount of scab. However, with various biologic and other factors, and a critical period for infection, temperature may prove to be more important in the field than it appeared to be under the controlled soil conditions tested.

Effect on viability. To determine the thermal death point of *A. scabies*, spores from a mature culture were soaked in water for thirty minutes and a very small portion of the spore suspension was put into 5 cc. of sterile water at the temperature under test. At ten- and twenty-minute intervals, portions were withdrawn and spread on cold agar plates to incubate. Repeated tests showed that spores germinated after having been exposed for twenty minutes at 83° but not after exposure at 85° C. They failed to germinate when exposed for ten minutes at temperatures higher than 90° C.

Since the spores of the scab organism resisted such high temperatures, it was decided to test the efficacy of the hot formaldehyde treatment. Experiment showed that scab spores on tubers were killed when immersed for two minutes in the hot formaldehyde solution (2 pints to 30 gallons of water) at 53° C. It also seemed desirable to test the efficacy of the acidulated mercuric chloride treatment for killing spores, because, if successful, its use would obviate the difficulty of the heating required with the formaldehyde treatment. The formula found effective by Cunningham (1) for killing sclerotia of *Rhizoctonia solani* was used. This method consists of soaking the tuber in a 1–1000 solution of mercuric chloride in water, to which has been added 5 cc. of commercial hydrochloric acid per liter. The tubers are kept moist for 24 hours after soaking. Experiments by the writer showed that this method was equally effective in killing spores of *A. scabies* and Rhizoctonia sclerotia.

Oxygen Requirements

Experiments were made to obtain more information on the relation of oxygen to the germination of the spores, the vegetative growth and fructification of *A. scabies*, because the pathogenicity of this soil fungus and the development of scab may be modified by the available oxygen in the soil.

Effect on germination and growth. A simple preliminary experiment suggested the strongly aerobic qualities of *A. scabies*. Six agar slants were inoculated with a spore suspension of the organism. Three tubes were sealed, and three were loosely plugged with cotton. The very faint growth which appeared after 30 hours in the sealed tubes was a striking contrast to the abundant development in those which were loosely plugged. No further increase occurred in the sealed tubes during 30 days. Apparently the available supply of oxygen had been exhausted during the first 30 hours. Another experiment, using the Büchner tube apparatus with alkalinated pyrogallic acid, demonstrated that the spores of *A. scabies* do not germinate in the absence of oxygen.

The relation of pure oxygen and nitrogen gas to the germination of the spores of the scab organism was also tested. These gases were allowed to displace the air in culture flasks, and then the flasks were sealed. No spores had germinated in 30 days in either gas, but, when exposed again to air, those which had been in pure nitrogen germinated and grew well. The spores which had been in oxygen were dead.

The effect of very limited and measurable amounts of oxygen on spore germination was also studied. Partial pressures, ranging from 0 to 29 inches, were obtained in test tubes containing agar slants which had been evenly inoculated with a spore suspension. Fresh inoculum was spread on the surface of the slants at the time the experiment was begun. The tubes

were fitted with rubber stoppers and glass tubes, and then sealed by heat after the air had been exhausted. By repeated experiment the results shown in table 2 were obtained. At zero partial pressure the spores required all the oxygen present in a 25 cc.-test tube to germinate and produce a very scant growth, while at a partial pressure of about 29 inches there was no visible sign of germination when examined microscopically. At a partial pressure of 28 inches short germ tubes appeared on some spores, but most of them did not germinate. The growth was very meager at a partial pressure of 27 inches. At partial pressures 25, 20, 15, 10, and 5 inches, a gradual increase in surface growth was evident over that obtained at a partial pressure of 27 inches, and increasingly higher percentages of spores also germinated at the lower partial pressures. The growth in the control tubes, which were loosely plugged with cotton, was in striking contrast to that at a partial pressure of 0 inches.

Effect on maturity. The relation of a limited amount of oxygen to vegetative growth, fructification, and especially to maturity (the darkening process of aerial hyphae) of *A. scabies* was investigated. Three inoculated petri dish cultures were placed in a large desiccator, a quantity of alkalinated pyrogallic acid calculated to absorb nearly all the oxygen was put at the base, and the cover sealed. White vegetative growth became evident in the control cultures in 24 hours and continued to increase, and on the fourth day the hyphae darkened rapidly. The test cultures grew as well as the controls up to the third day, but they failed to darken or to develop further during eleven days. When air was admitted, the retarded cultures grew quickly. In twenty hours they had darkened very noticeably, and by forty-eight hours were almost as dark as the controls. No detached spores and segments of mycelium were evident when the retarded cultures were examined on the eleventh day, but a later examination of the mature cultures revealed both the spores and segments common to ripe hyphae.

Relation of quantity of oxygen to partial pressure. While observing the germination and growth of *A. scabies* at the high partial pressures, it was suggested that the fungus might not be able to obtain the small quantity of oxygen left in the test tube; that is, the quantity of oxygen and not the partial pressure becomes the limiting factor to growth. No data on this point were found in the literature. Accordingly, a partial pressure of about 29 inches was obtained in two 25 cc. test tubes containing inoculated agar slants, and also in two 11,550 cc. bottles each containing one inoculated agar slant. The partial pressure was tested both in the large and small containers after ten days and found to have remained at about 29 inches. At this time, meager growth, similar to what had occurred in the test tubes at a partial pressure of 10–5 inches (see table 2) could be seen on the slant contained in each of the large bottles. The spores in the small test tubes at

the same partial pressure failed to germinate. The experiment was repeated three times with consistent results. These results suggest that this fungus is able to obtain practically all the oxygen left in a large volume, regardless of partial pressure. Therefore, it appears that quantity of oxygen, and not partial pressure, is the limiting factor to germination and subsequent development of *A. scabies*.

These studies show that *A. scabies* is very dependent on an abundant oxygen supply for quick germination and rapid vegetative development, and also for the ripening process, which means the darkening of the aerial

TABLE 2.—*The effect of various quantities*[a] *of oxygen on spore germination and growth of Actinomyces scabies*

Partial pressure in inches	Growth[b] and germination of spores after				Remarks
	24 hrs.	48 hrs.	72 hrs.	15 days	
29	0	0	0	0	No germination
28	0	0	0	0	Short germ tubes on a few spores
27	1	1	1	1	Extremely meager growth
25	2	2	2	2	More spores germinated; meager growth
20	2	2	2	2	*do*
15	2	2	2	2	Better germination
10	3	3	3	3	Faint growth
5	3	3.5	4	4	Faint growth
0	5	6	6	6	Better growth
Control[c]	25	75	95	100	Excellent ashen colored growth after 72 hours

[a] Obtained by partly exhausting the air from a 25 cc. space in test tubes; barometer reading 72.6 cm.

[b] Estimated relative value of growth at 26° C.

[c] Cultured in loosely plugged test tubes.

hyphae accompanied by easy disintegration of hyphal segments and spores for inoculum. No definite data have been secured relative to oxygen requirements in the soil, or the oxygen-supplying ability of soils of various types and moisture contents; but it seems quite probable that lack of sufficient oxygen is often an important factor in limiting sporulation, spore germination and vegetative development of the pathogene in the soil. This would then affect the ability of the parasite to cause infection and may help to explain why scab is not so prevalent in wet soils as in drier ones.

Moisture Relationships

From 1920 to 1923 the writer (13, 14) found that very little scab occurred when the soil was kept constantly very moist, but abundant scab

appeared in the same soil kept very dry. It was shown that the control obtained in the moist soil was not due to increased acidity, because the dry soil proved slightly more acid than when wet. It was thought that the pathogene might not sporulate as well in saturated soil air, or, possibly, the germination and growth might be delayed by the excess soil moisture around the spores.

Growth on solid media. Equal portions of spore suspension were spread on nutrient agar in petri dishes and cultured in various relative humidities, ranging from 1 to about 95 per cent. Saturated solutions of the following chemicals were used to obtain the relative humidities indicated: H_2SO_4, less than 1 per cent; LiCl, 7.16 per cent; $CaCl_2$, 25.94 per cent; NaOH,

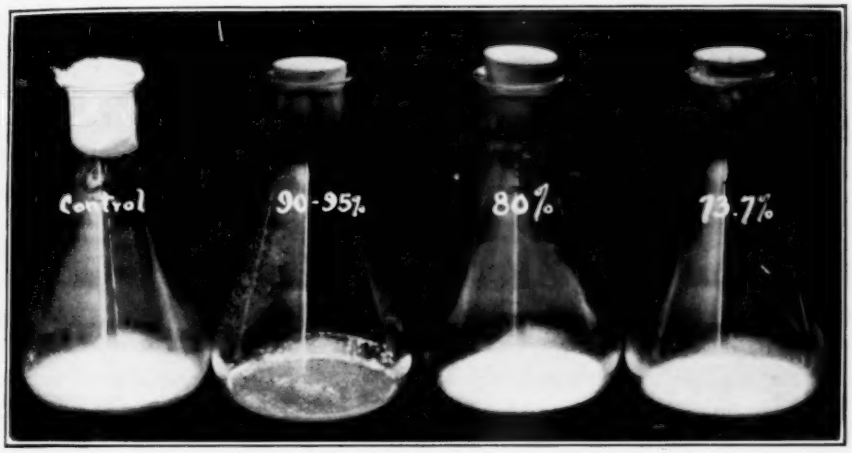


FIG. 1. Growth of *A. scabies* at various relative humidities. Note delayed germination and growth in second flask.

30.72 per cent; $AlCl_3$, 37.1 per cent; $Cu(NO_3)_2$, 45.7 per cent; NaCl, 73.7 per cent; $NaNO_3$, 80 per cent; K_2SO_4, 89.7 per cent; distilled water 90 to about 95 per cent. Owing to the surface condition of the agar when dried too quickly, no satisfactory data were obtained by this preliminary trial, although it was evident that excess water on the substrate delayed growth. In another experiment, saturated air was led successively through distilled water, and a saturated solution of sodium nitrate and sodium chloride. Between each of these solutions, and connected with them, was a 200 cc. flask containing 10 cc. of nutrient agar and 0.5 cc. of spore suspension over the surface of the medium. Within 15 hours vigorous growth appeared in the flask whose humidity was controlled by sodium nitrate, but not until after 60 hours in the flask whose humidity was determined by distilled water (figure 1). Final inspection showed that the cultures retarded by excess

moisture still lagged in development behind those getting an optimum start. This experiment was repeated a number of times. It is suspected that the film of water around the spores remained longer at the higher humidity than at the lower ones, possibly limiting the oxygen supply. It was repeatedly observed that dry spores put into water tend to remain on top, but, if the liquid is shaken frequently and the spores kept immersed, germination may be greatly delayed. If left, however, the spores on top germinate, while those immersed usually remain dormant. It was found that optimum conditions, good vegetative growth can be secured in 24 hours, spore suspension evenly over the surface of agar slants in loosely plugged test tubes, and incubating them in an upright position at 27° C. Under optimum conditions, good vegteative growth can be secured in 24 hours, and a fair degree of maturity (as indicated by the dark color of the aerial growth) in 48 to 60 hours, but this may be greatly postponed by less favorable conditions, even at optimum temperature.

Viability of spores. Studies were made to determine the effect of relative humidities at various temperatures on the viability of the spores of *A. scabies.* A small amount of the aerial hyphae from mature cultures was placed in small sterile phials. These were lightly plugged and placed in sealed jars. Relative humidities of approximately 95, 80, 46, 37, 7 and 1 per cent were obtained by using distilled water, saturated solutions of sodium nitrate, copper nitrate, aluminium chloride, lithium chloride, and concentrated sulphuric acid, respectively. A series of each was incubated at 30°, 26° and 12° C., respectively. Viability tests were made each month. After six months, the spores germinated well, regardless of temperature or humidity.

Soil moisture and increase of inoculum. The amount of inoculum in the soil during the susceptible period of the tuber is an important factor influencing the amount of infection. The soil dilution and plate method was used to determine the relative effect of moist and dry soils on the increase of inoculum. Eight grams of finely-sifted, oven-dry soil was put into a 200 cc. flask and sterilized with dry heat. Five cc. of spore suspension was put into each flask, and after the moisture in the inoculum had diffused, the soil was thoroughly mixed, then various additional amounts of sterile water were pipetted into the flasks and distributed as evenly as possible. The culture flasks were in triplicate in each series. Provision was made for aeration, and the soil cultures set to incubate in a high humidity at 26° C. for 30 days. Dilutions were made by adding enough sterile water to the flasks so that each would finally contain 100 cc. The contents of each flask were then emptied into 900 cc. of sterile water. After shaking and allowing the contents to settle for 30 seconds, a 100 cc. portion was withdrawn from a uniform depth and introduced into another 900 cc. of water. Further

dilutions of 10–90 cc. were made, and a 1 cc. portion of the final dilution was pipetted into each of four culture plates. This method of dilution was used to reduce the error arising from the possible unequal distribution of the inoculum added to the culture flasks, and the very high probable error that was found by repeated trials to exist when only 1 cc. portions were withdrawn from the first dilution. Even this method leaves much to be desired, as the probable error is still high.

The results shown in table 3 indicate that the inoculum failed to increase in the driest soil (6.25 per cent), but at 10, 15, 21.2 and 27 per cent soil moistures it continued to increase until it reached about five-fold its original amount. A sudden decrease is observed at 33.7 per cent soil moisture. Possibly this is on account of the lack of fructification, caused by the hyphae in the very moist soil retaining their vegetative state and failing to break up into segments. The increase at 37 per cent soil moisture is abnormal since in this excessively wet soil the spores were carried by free water to the surface, where they germinated, and where sporulation later occurred.

TABLE 3.—*The effect of soil moisture on increasing the inoculum of Actinomyces scabies cultured in a sandy loam*

Soil moisture in per cent	6.25	10	15	21.2	27	33.7	37
Average no. colonies obtained from diluting 80 grams soil	7.1	20.2	31	38	34	17	45[a]
Per cent increase over control (7.1)		184.5	336.6	435.2	378.8	139.4	533.8

[a] This abnormal increase is the result of surface growth on the excessively wet soil.

Soil moisture and scab development. According to the results of the previous experiment and the behavior of the pathogene on nutrient agar, favorable conditions for production of abundant inoculum, and for subsequent growth, would not be found either in excessively dry or in very moist soil but in one of intermediate moisture content. However, the writer (13, 14) found that a maximum amount of scab may develop in a very dry soil.

In one instance tubers developed to maturity in soils having about 4 per cent of moisture by weight (oven-dry basis). The water was led by a tube below the surface soil, in which the tubers formed, to the roots of the plant. It was thought sufficient moisture transpired from the tuber in so dry a soil to enable the spores adhering closely to the surface, or about the lenticels, to germinate, and that the germ tubes grew toward the source of

moisture. Perhaps increased infection of the tuber in dry soils may be due to a hydrotropic response, or the lenticel structure and hydrotropism both may affect it. When once established on the host, the pathogene would develop regardless of the lack of soil moisture, and a dry soil would allow good aeration. Lutman (8) states that scab may originate at any place on the surface of the tuber, and that it most frequently occurs at the lenticels. From extensive observation, the writer believes that the lenticels are the principal infection courts, and are the centers from which the typical scab pustules develop. It is common knowledge that the proliferation of the lenticels of potatoes is increased in wet soils, and it is quite possible that there is a close correlation between early lenticel growth in dry soils and susceptibility to the scab pathogene.

The possibility of the growing tuber exerting a chemical stimulus on the spores of the scab organism, or on the germ tubes, was not overlooked. From repeated experiment there was no indication that the tissue of young or old tubers, or of potato leaves, stimulated the germination of the spores of *A. scabies*.

The writer (13,14) twice reported a critical period of susceptibility to scab infection. This occurred during the first ten days of tuber development. With a critical period for infection, the relation of soil moisture to scab development becomes very important. Young tubers may escape infection during this period if excessive soil moisture or other unfavorable factors inhibit the growth of the pathogene, and, likewise, an effective means of control must exert its greatest influence at this time.

Relation of Soil Reaction to Germination and Growth

Gillespie (4) concluded from a study of several cultures of *Actinomyces scabies*, that a pH of 5.2 is about the lower limit for growth. Slight development occurred in few instances at pH 4.8.

The effect of hydrogen-ion concentration on the growth and spore germination of the strain used in these experiments was determined. A wide range of pH was obtained by adjusting Czapek's synthetic dextrose-nitrate solution, as recommended by Karrer and Webb (7). Twenty cc. of sterile solution thus made up were measured into 200 cc. pyrex flasks, loosely plugged, then 1 cc. of spore suspension was added, and the flasks set to incubate at 26° C. Electrometric determinations were made of the initial reaction of each series, with and without inoculum, and this was repeated in ten days in the acid series, and in 48 hours in the alkaline series, in order to note any change in reaction. The solutions containing inoculum soon became alkaline, depending on the rate of growth. Notes were taken on the time required for growth to appear, its relative progress and final develop-

ment. Each series was in triplicate, and the whole experiment was repeated three times. The results are summarized in table 4.

Evidently pH 5.36 is about the limiting acidity for germination of the strain of *A. scabies* used in the test, since microscopic examination at the end of 20 days showed no evidence of spore germination at this reaction. Possibly other pathogenic strains of *A. scabies* have a different limiting pH value for germination. A few slow growing colonies appeared on the seventh day at pH 5.56. At pH 6.95 to pH 9.82[4]–9.05, visible growth occurred in 48 hours. For the first three days there was a constant tendency for earlier and better growth above than below pH 8.30, but after this period the cultures growing in the media with initial pH ranging from 6.48 to 8.68

TABLE 4.—*The estimated growth progress of Actinomyces scabies, at 26° C, on Czapek's solution at different hydrogen-ion concentrations.*

Culture	pH value of medium		Growth progress at various day intervals					
	Initial	After 10 days	2	3	4	5	7	20
1	4.52	4.50	0	0	0	0	0	0
2	4.70	4.70	0	0	0	0	0	0
3	4.87	4.86	0	0	0	0	0	0
4	5.21	5.20	0	0	0	0	0	0
5	5.36	5.37	0	0	0	0	0	0
6	5.56	5.83[b]	0	0	0	0	tr.	4[c]
7	5.69		0	0	tr.	1	2	6
8	6.08		0	1	3	7	8	8
9	6.48		0	1	4	8	10	10
10	6.85		0	2	6	9	10	10
11	6.95		tr.	2	6	9	10	10
		After 48 hours						
12	7.16		tr.	2	7	9	10	10
13	7.56	7.51	tr.	3	7	9	10	10
14	7.76	7.66	tr.	3	7	9	10	10
15	8.38	8.30	1	4	7	9	10	10
16	8.68	8.51	2	6	8	9	10	10
17	9.31	8.80	2	6	8	9	9	9
18	9.43	8.88	2	6	8	8	8	9
19	9.82	9.05	2	6	8	8	8	8
20	10.21	9.34	0	0	2	4	5	7

[a] 0 = no growth; tr. = trace; 1 = faint; 2 = plainly visible; 4 = fair; 6 = good; 10 = excellent.

[b] Determination made from growing culture.

[c] Few well developed colonies.

[4] The first figure is the initial reaction, the second that obtained after 48 hours.

seemed to have made more vegetative growth and to have fructified more abundantly than those above pH 8.68. Germination was slow and final growth poor above pH 10.2–9.34. No attempt was made to determine the maximum pH for germination because of the difficulty of holding the high alkaline solutions constant and the probable effect on growth of the large amount of potassium hydroxide. It would appear, however, that this point is not far from pH 10.

Available food, soil, aeration, moisture, and other biological factors, as well as soil reaction, must influence the amount of inoculum in the soil, and the amount of scab which develops. However, it does not appear that a soil reaction which is the limiting point for germination of the inoculum will completely inhibit development of scab. This might be because the growing tuber has a reaction favorable to germination and growth of *A. scabies*. Weiss and Harvey (19) found the reaction of the potato tissue to vary from pH 6.5 to 6.61. Consequently, the acidity about the closely adhering inoculum might be decreased enough to allow germination of the spores and infection of the host. If so, subsequent development would be largely determined by the reaction of the host tissue. Possibly this suggests why Martin (10) obtained scab on 20.6 and 89.6 per cent of the tubers in a soil of pH 4.2 and 4.8, respectively.

Weiss and Harvey found that tissue affected with potato wart was more acid (pH 6.0) than the adjacent sound tissue (pH 6.5). The writer determined electrometrically the reaction of scab pustules. Very scabby young potatoes grown in a soil having a reaction of about pH 5.4, were washed in distilled water, the scab pustules removed, finely pulverized, and the material diluted with distilled water. Sound tissue was treated likewise, and determinations made as soon as possible. The pH of the sound tissue was about 6.5, while that of the scab pustules was about 7.2. This increase in alkalinity agrees with what happens in liquid cultures and supports the previous suggestion that the development of *A. scabies* on infected tubers is largely independent of soil reaction.

The Relation of Green Crops to the Control of Potato Scab

The popular practice of plowing down green crops, particularly rye, to reduce the severity of scab is based largely on the assumption that decomposition of the green material increases soil acidity. Since consistent evidence for such belief seemed to be lacking, the writer tested the efficacy of the method in a well checked field experiment.

The effect of green rye plant material on soil reaction. A small, nearly level, thoroughly infested plot of fertile silt loam, with an initial pH ranging from 4.91 to 5.4, was chosen for the test. Twelve rows, of 12 hills each, were planted, test rows and controls alternating. Two and one half pounds

of succulent green material was mixed with the soil, and this put back into the excavations, which were 14 inches in diameter and 7 inches deep. This proportion of green material amounts to about 50 tons per acre. The soil in the control hills was forked to make it as loose as that under test. A disinfected set of the cobbler variety was planted 5 inches deep in each hill. The soil about each plant was not disturbed during the growth period. The rye was almost completely decomposed by harvest, 80 days after date of planting (May 24). The reaction of the soil, taken from representative hills at depths of 2, 4 and 6 inches, was determined electrometrically on June 19, July 2, and July 22. Samples were taken from the same hills each time, so that any change in soil reaction resulting from the decomposition of the green material might not be confused with soil heterogeneity. One hundred and thirty-two samples, of which 24 were checks, were tested, and three determinations made from each sample.

The object of this experiment was to note any change in soil reaction caused by the green rye during the early growth period of the potato crop, and particularly during the critical stage for scab infection. Therefore the reaction of the soil was not determined after 58 days. The data indicated

TABLE 5.—*The effect of varying amounts of green rye on the soil reactions and on the relative number of bacteria and fungi*

Parts of rye to soil	Interval after which pH was taken					Initial pH	Maximum pH increase	No.[a] colonies	
								Bacteria[b]	Fungi[c]
	Series 1								
	10 days	20 days	28 days	36 days	78 days				
	pH	pH	pH	pH	pH				
1–5	8.99	7.56	7.25	7.10	7.04		1.82		
check	7.22	7.20	7.13	7.14	7.14	7.17			
	Series 2								
	7 days	15 days	33 days	75 days					
	pH	pH	pH	pH	pH				
1–3	8.82	8.22	5.82	5.07			3.60	200	70
1–7.5	7.70	7.49	5.24	4.98			2.48		
1–10	7.37	5.76	5.37	4.97			2.15	134	33
1–15	6.89	5.73	5.24	5.24			1.67		
1–20	6.67	5.51	5.27	5.22			1.45	16	26
check	5.31	5.25	5.22	5.19		5.22			

[a] In millions per gram of rye soil mixture, oven dry.
[b] Dilutions made after 20 days.
[c] Dilutions made after 10 days.

a slight decrease in soil acidity. This conclusion agrees closely with that of Stephenson (16) who found that in nearly every case the acidity was decreased where soy beans, oat straw, green rape, and blood were used alone and in combination in the soil. Oat straw had the least effect in decreasing the acidity, while the highly nitrogenous material had the greatest.

Another study was made in the laboratory to determine the effect of the decomposition of green rye plants on soil reaction. A quantity of leaves from green rye plants was finely ground. This was mixed in varying amounts with finely sifted, unsterilized garden soil, then moistened with distilled water and put into loosely plugged liter flasks. All samples were approximately at optimum moisture content when completed, and the soil was loose in the flasks. The initial reaction of the soil, and also of the green material used, was determined electrometrically. The flasks were kept at room temperature and the soil reaction determined at intervals during 80 days. The number of fungi and bacteria developing in the soil of the various flasks was obtained by the usual dilution-plate method, and a comparison made on an oven-dry basis per gram of soil.

An analysis of the results in table 5 shows that the pH is decidedly increased in all the soils mixed with green rye-plant material, especially during the first ten days, and that this sudden rise is succeeded by a slow but steady decline during the second period. After about 30 days the pH values approach a level approximating the initial reaction. This is especially noticeable in the soils containing the smaller proportion of plant material. The increase in pH is, in general, proportional to the amount of plant material added to the soil, and varies from 1.45 to 3.60. In most cases, even after 30 days, this advantage is maintained. After 30 days, and continuing to 75 days, all the samples return to the initial reaction and, when the last determinations were made, only those samples receiving the larger proportions (1–3, 1–7.5, 1–10) of rye were slightly more acid than the untreated soils.

Since the determinations were made on the rye-soil samples after fermentation began, the increase due to the reaction of the rye itself was unknown; therefore a separate experiment was necessary to obtain this information. The initial pH of the green rye was 6.82, and that of the soil 5.12. The data show that the pH of the rye-soil mixture was increased very slightly by adding the green rye. The largest proportion of rye to soil (1–5) increased the pH 0.36, while the 1–10, 1–15, 1–20 proportions gave increases of 0.20, 0.18 and 0.17, respectively. After an 8-day incubation period the increases in pH of these same mixtures were: 1–5, 1.71; 1–10, 0.94; 1–15, 0.80; 1–20, 0.71. Apparently, then, nearly all the increase in pH of this and the previous test was the result of fermentation.

Two suggestions are given for the consistent increase in pH of the rye-treated soils; either the rye may favor certain microorganisms which decompose it and liberate ammonia for a time, or certain alkaline by-products of metabolism may be secreted by the fungi and bacteria themselves. Doryland (2) found that nitrification begins when the more easily available carbohydrate energy is exhausted, and states that the ammonia and nitrates which have been accumulated by the decay-producing organisms during this period are later available for nitrification. Waksman (18) states that the molds are not nitrifiers but they actively decompose organic matter, taking energy first from the available carbohydrates and then from protein molecules, in which case ammonia is liberated. He also points out how ammonia may be liberated upon autolysis of the mycelium of these molds. Neller (12) found that certain pure cultures of bacteria caused the accumulation of much more ammonia than some of the more active soil fungi (*Aspergillus niger, Trichoderma* sp., and *Zygorhynchus* sp.) and that there was an indication of symbiosis in mixed cultures of certain soil bacteria in increasing the accumulation of ammonia.

It is possible that the large amount of carbohydrate energy in the rye permitted abundant and quick growth of microorganisms, with a corresponding nitrate depletion of the soil, and that, upon the sudden exhaustion of the carbohydrate energy supply and the consequent setting in of the nitrification process, certain more favored bacteria and fungi actually cause the liberation of ammonia.

It was suggested that the increase in pH might be due to the alkaline by-products secreted by one or more predominating species of microorganisms. It has been shown that *A. scabies* greatly increases the pH of the culture media tested. It also is well known that other microorganisms produce acids and alkalies, depending on the chemical nature of the substrate.

A plate count of the microorganisms was made from dilutions of the soil of Series 2 (see table 5). In the dilutions made 10 days after mixing the rye and soil, the number of fungi on the plate made the count of the bacteria too uncertain to record, but, when made after 20 days, the plates were fairly free from fungi, and bacteria predominated. A heavy growth of molds (*Mucor racemosus* appeared to predominate) occurred in all flasks containing the rye-soil mixture. This took place two days after mixing; and in ten days it had declined, the soil taking on a moist, slimy appearance, which indicated bacterial activity. No further change was noted and no more soil dilutions were made. It is suggested that the fungal-bacterial succession referred to here may be associated with the rise and decline of pH noticed in these rye-treated soils.

The effect of green rye plant material on scab development. Figure 2 shows the amount of scab developing on the tubers in rye-treated and untreated soils of the field. Each pile represents the complete yield from a row, and these are arranged as they grew—treated and untreated rows alternating. No difference between the test lots and the controls can be noticed: all are badly scabbed. These results differ from those of Millard (11), who obtained good control by a liberal application of green rye; and, since the data on soil reaction could not explain the results, a preferential food theory was offered. This theory assumes that the scab pathogene, being normally a soil saprophyte, prefers green rye to the living potato tissue. The hypothesis may be tenable. The writer advances another possible explanation, and this will be referred to in connection with the antibiosis of certain bacteria and fungi to *A. scabies*.

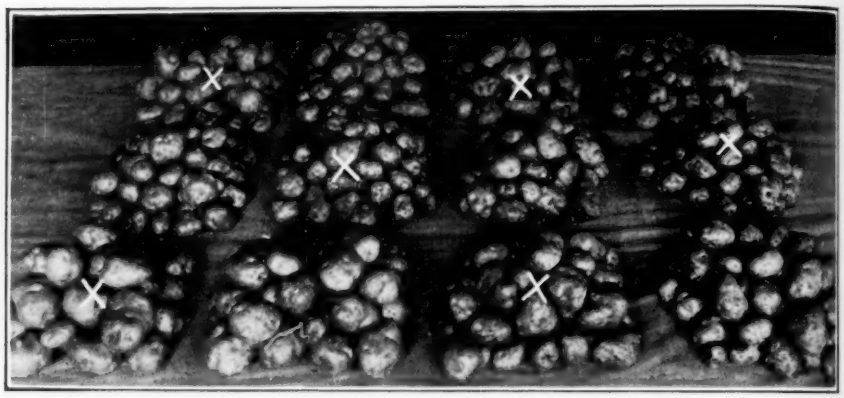


FIG. 2. The piles of tubers marked X are from soil (pH 5 to 5.4) treated with 50 tons per acre of green rye; remainder from untreated soil. Note uniformly large amount of scab in all piles.

The data from the field experiment showed that the decomposition of 50 tons of green rye per acre caused no noticeable increase in soil acidity during the 58-day period and failed to prevent the development of scab. The laboratory experiment showed that it required a much larger quantity than this to increase the acidity of the same soil, even slightly, at the end of 75 days. It is interesting to recall that the initial reaction of this soil varied from pH 5.0 to 5.4, which is very near the limiting reaction for growth of *A. scabies* in pure culture. The acid-producing effects of green rye on other soil types was not tested. However, these results strongly suggest that an abnormally large amount of rye would be required to increase the acidity of less acid soils to an effective point for scab control. Therefore it is the

opinion of the writer that the use of green rye as a practical means of increasing the soil acidity for the control of potato scab is not feasible. If control can be obtained by its use in some soils, it may be that certain microorganisms of the greatly increased soil flora are antibiotic to *A. scabies.*

Cultural Relations of Certain Bacteria and Actinomyces scabies

While testing the stimulatory effects of potato leaf- and tuber-tissue on the germination of the spores of *A. scabies,* it was frequently observed that the spores failed to germinate on certain slides overrun with bacteria. Further examination showed that the lack of germination was often associated with the kind of bacteria present, therefore possibly a result of toxicity or a too highly acid condition.

Effect of mixed cultures on the pH of the substrate. To study the effects of association of bacteria and *A. scabies* in culture, a number of pure cultures of bacteria were secured from various sources, chiefly from the soil. These were numbered as represented in table 6. Culture 21 is *Bacterium fluorescens;* 28, *B. mycoides;* 24, *B. cereus.* These were obtained from H. J. Conn, New York Agricultural Experiment Station. Culture 22 is *B. vulgatus;* 23, *B. megatherium;* 25, *B. mycoides;* 27, *B. mesentericus,* all of which were furnished by O. B. Williams, University of Texas. Czapek's dextrose nitrate solution was used for a medium. This was sterilized and adjusted to the required pH values by the addition of one-tenth normal potassium hydroxide. Pyrex flasks were used, and to each was added 20 cc. of the medium, and inoculum consisting of 0.1 cc. of a spore suspension of *A. scabies,* and the amount of bacteria adhering to the small loop of a transfer needle. Each culture was made in triplicate and incubated at 26–27° C.

The results given in columns 2 and 7, table 6, indicate that nearly all the strains of bacteria are strong acid producers. The reactions listed in column 2 give an idea of the acid-producing ability of each pure culture of bacteria when grown alone. Cultures 21 and 29 are fairly strong acid producers, although in Series 1 the time during which they were incubated is too short to ascertain the final effect in this respect. The reactions in column 6 indicate the strong alkali-producing ability of *A. scabies* during six days, when the initial pH of the medium is 7.3. This quality is also shown, in columns 9 and 11, in those cultures where the Actinomycetes are growing vigorously, apparently having secured control by neutralizing the acid from the bacteria. Evidently *A. scabies,* when grown alone or with certain bacteria, grows and fructifies exceptionally well at pH 8.0 to 8.7. Most of the cultures of bacteria allow a good final growth of *A. scabies* if the initial pH of the medium is sufficiently high. Bacterial cultures 11, 15, 21 and 29, however, always prevent the growth of the Actinomycetes. In the case of

cultures 11, 21 and 29, the growth is prevented by a too acid condition of the medium, *i.e.*, pH 4.23, 4.43 and 5.29 respectively. These cultures have reduced the pH from 7.3 to these low values in six days, while most of the other strains allowed the Actinomycetes to increase it to about 8.7. Culture 15 is outstanding, because it always prevents the growth of *A. scabies*,

TABLE 6.—*The effect of pure cultures of bacteria growing alone, and also with A. scabies, on the pH value of Czapek's solution*

Culture no.	Series 1					Series 2				
	Bacteria initial pH 7.3	Bacteria and *A. scabies* initial pH 7.3				Bacteria initial pH 6.87	Bacteria and *A. scabies* initial pH 5.7			
		Growth: after 3 days	pH	Growth: after 6 days	pH		Growth: after 6 days	pH	Growth: after 11 days	pH
1	6.53	Good	7.41	Ex.[a]	8.67	.....	.....	.....	.....	.....
2	6.44	do	7.68	do	8.72	.....	None	5.02	None	5.41
4	6.12	do	6.48	do	8.75	.....	.....	.....	.....	.....
5	6.13	do	7.12	do	8.73	.....	None	4.91	None	5.59
8	6.61	do	7.33	do	8.72	.....	.....	.....	.....	.....
10	6.99	do	7.67	do	8.69	.....	.....	.....	.....	.....
11	6.05	Trace	4.34	None	4.23	.....	None	4.77	None	4.57
12	6.86	Good	7.39	Ex.	8.73	.....	do	5.40	Ex.	8.04
13	5.67	do	7.80	Good	8.72	.....	.....	.....	.....	.....
15	6.78	None	6.63	None	7.28	.....	None	7.35	None	7.29
16	6.44	Trace	6.44	Ex.	8.72	.....	Trace	4.89	do	4.22
17	6.61	do	6.95	do	8.59	.....	None	4.57	do	4.60
19	6.30	Good	7.14	do	8.57	5.51	do	5.32	Good	8.14
20	6.89	do	7.17	do	8.57	.....	.....	.....	.....	.....
21	6.71	None	5.11	None	4.43	4.30	None	4.73	None	4.80
22	6.61	Good	7.49	Ex.	8.72	.....	.....	.....	.....	.....
23	6.81	Trace	5.43	Faint	7.36	.....	None	4.92	Edge	5.41
24	6.53	Good	7.61	Ex.	8.72	6.16	do	5.29	do	6.41
25	6.71	do	7.21	do	8.72	6.14	do	4.81	None	4.73
26	6.64	do	6.97	do	8.70	.....	.....	.....	.....	.....
27	6.69	Trace	7.39	do	8.79	5.66	None	4.89	None	5.03
28	6.53	Good	7.26	do	8.89	5.92	do	4.72	do	4.72
29	6.66	None	5.26	None	5.29	5.34	do	4.83	do	4.64
30	6.99	Edge	7.56	Pel.[b]	7.80	.....	Pel.	7.35	Edge	7.63
31	6.71	Fair	7.22	Fair	7.46	.....	None	5.27	Ex.	8.04
32	7.05	Good	8.52	Ex.	8.72	.....	.....	.....	Pel.	6.07
33	6.66	do	8.01	do	8.72	.....	Pel.	7.06	Edge	7.70
Check	7.70	do	8.51	do	8.72	.....	Fair	7.06	Ex.	8.40

[a] Excellent.

[b] Pellicle.

although it does not reduce the pH below a point favorable for growth of the fungus. When the initial pH of the medium is low (5.7), as in Series 2, many of the cultures of bacteria retard or prevent the growth of *A. scabies* by quickly increasing the acidity of the substrate, but some do not produce acid fast enough to accomplish this. This competition between the bacteria and the Actinomycetes makes the final outcome uncertain, as is shown by cultures 2, 5, and 23, in columns 9 and 11.

Further examination of table 6, Series 1, shows that some strains of bacteria increase the acidity of the medium much more when cultured with *A. scabies* than when alone. This action is well illustrated by cultures 11, 15, 21 and 29, in figure 3 and particularly by cultures 11, 21 and 29. The decrease in pH, as a result of association, ranges from 0.95 to 1.71. A parallel case is reported by Neller (12), who found that *Bacillus mycoides* and *B. vulgatus* produced more ammonia in mixed cultures than when alone, and that the same thing was true of the production of carbon dioxide by *B. subtilis* and *B. vulgatus*. Other instances of increased activity arising from the association of certain microorganisms are known.

Toxicity and compatibility in mixed cultures. Grieg-Smith (5) states that certain white-growing colonies of Actinomycetes secreted some product very toxic to *Bacillus vulgatus, B. mycoides* and also to *Penicillium* spp. and *Aspergillus* spp., and that the dark-growing[5] Actinomycetes were only feebly toxic to *B. vulgatus*. The writer has observed that *A. scabies* is, in general, very sensitive to the secreted products of many molds and bacteria, some of which prevent its growth. *Rhizopus* spp. have been consistently very toxic. Species of *Penicillium* and *Aspergillus* usually appear to be toxic to *A. scabies*, but this condition often develops later into one of compatibility, especially on solid media. Some of these cases of toxicity are probably associated with competition for food and with an unfavorable substrate reaction. The competition for favorable substrate reaction at the minimum point for growth is well illustrated by *A. scabies* and bacterial cultures 19 and 24, Series 2, table 6, and in figure 3, part 2. Bacterial culture 15 evidently secretes another kind of toxic substance, which, while not too acid, prevents the growth of *A. scabies*.

Further investigation of the association of *A. scabies* and the various strains of bacteria cultured in solution (table 6) was made on solid media. Potato dextrose agar slants were evenly inoculated with 0.2 cc. of spore suspension of *A. scabies* and then inoculated for 24 hours. By that time growth was quite evident. Then 0.2 cc. of sterile distilled water was added to the very bottom of each tube, and this was inoculated with a small amount of bacteria adhering to the loop of a transfer needle. Care was taken to add

[5] *Actinomyces scabies* belongs to this group.

nearly equal quantities of the bacterial inoculum to the water at the bottom of the tube only, and to keep the tubes upright. Striking differences in

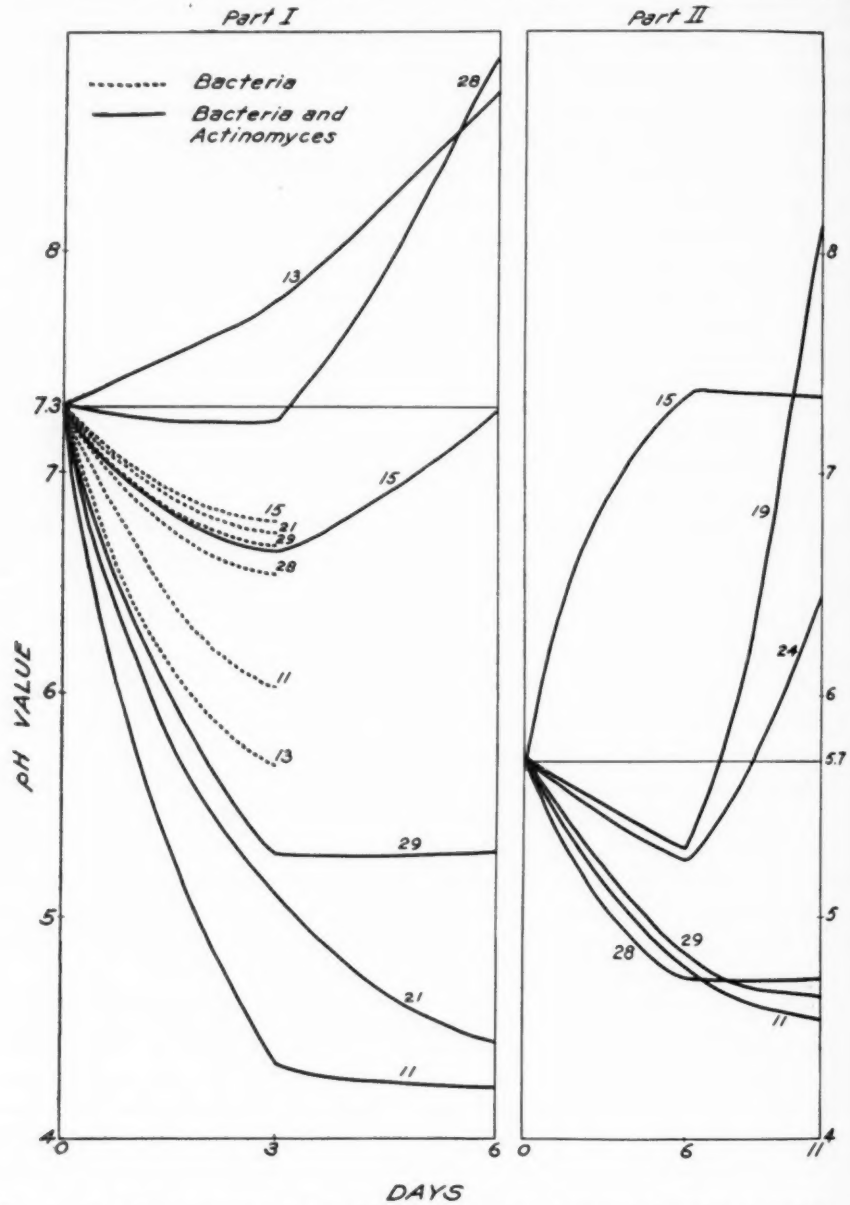


FIG. 3. The effect of pure cultures of bacteria growing alone and also with *A. scabies*, on the pH value of Czapek's solution. (Each culture is designated by the number just above the pH curve; the numbers correspond with those in table 6.)

growth were evident in a few days. A number of the strains seemed more or less compatible. As happened in the liquid media, bacterial strains 11, 15, 21 and 29 were sufficiently toxic to *A. scabies* to destroy the growth after it was visible. Cultures 2, 5, 12, 28 and 30 also were very toxic, although they had not shown this in the liquid media. The phenomenon is not unlike the activity of a lytic principle.

It is quite possible that the same degrees of compatibility between organisms and the same toxicity demonstrated on artificial media may also occur naturally in the soil. This is a problem of the biological balance in the soil and therefore may be of particular importance in determining the pathogenicity of *A. scabies*.

SUMMARY AND CONCLUSIONS

1. A study has been made of the effect of temperature, moisture, aeration, and substrate reaction on the germination, growth and fructification of *Actinomyces scabies*, with particular reference to its pathogenicity. The relation of green rye to the control of potato scab has been studied, and its effect on soil reaction and on the increase of soil flora has been observed. A brief study has been made of some cultural relations between *A. scabies* and some common soil bacteria.

2. *A. scabies* grows within a wide range of temperature (8 to 38° C). Good growth and maturity occur between 13° and 32° C., and the optimum temperature is about 27° C. Therefore, under average field conditions in most potato growing areas, it appears that temperature, as it affects host and pathogene only, cannot be a very important factor in the scab problem. The spores survive temperatures up to 90° C. (moist heat) for ten minutes.

3. *A. scabies* is a strong aerobe. The spores will germinate with an extremely small supply of oxygen, but a large amount is required for subsequent development. Maturity, as indicated by dark aerial hyphae, will not take place in the absence of oxygen. Amount of oxygen, not partial pressure, is the limiting factor for germination and growth.

4. It was found that the germination of spores of *A. scabies* on nutrient agar was greatly retarded by a lagging film of excess water. The inoculum of *A. scabies* appeared to increase best at a soil moisture content about optimum for plant growth.

5. The limiting acid reaction for germination of the spores of the strain of *A. scabies* used was found to be about pH 5.3. Germination occurred most quickly at about pH 8.5, and an optimum development took place at this point. Severe scab may be expected in soils ranging from a strongly alkaline reaction to at least pH 5.4, owing to the higher pH of the tuber and a strong tendency of the pathogene to make its habitat (scab pustule) alkaline.

6. Green rye plants, applied in the field at the rate of 50 tons per acre, showed no tendency to reduce scab in a thoroughly infested soil of pH 5 to 5.4; and there was no noticeable increase in the soil acidity during a 58-day period.

7. In the laboratory there was a very slight increase in acidity of the rye-treated soils after 75 days, but only in those receiving large proportions (1–3, 1–7.5, 1–10) of green rye. These results, and those obtained in the field, indicate that the use of green rye does not control potato scab by increasing the soil acidity.

8. When pure strains of bacteria and *A. scabies* were cultured in the same solution, certain strains of the former made the solution too acid for the germination of the latter. Certain cultures of bacteria produced more acid when grown with *A. scabies* than when grown alone.

9. A strain of bacteria isolated from the soil, and cultured in Czapek's solution, inhibited the development of *A. scabies* by some means other than excessive acidity.

10. Nearly all the bacterial strains used in this study made conditions more or less unfavorable for the growth of *A. scabies* on a potato-dextrose agar.

11. When scab is controlled by green rye crops on some soils, it is suggested that the antibiotic qualities of certain predominant soil microorganisms influence the development of *A. scabies*.

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BLACK-LEAF OF PEAS CAUSED BY FUSICLADIUM PISICOLA N. SP.

MAURICE B. LINFORD[1]

In the summer of 1921 a leaf-spot disease was observed in fields of canning peas (*Pisum sativum* L.) at Smithfield, Cache County, Utah, where it was causing considerable leaf destruction in peas at canning age. Although peas had been grown in this locality since 1918, this disease had not been reported before. The disease was clearly distinct in appearance from any known to affect peas, being characterized by spots of sooty blackness on the lower sides of leaves and on petioles and tendrils. Microscopic examination revealed the presence of two fungi or two sporulating forms, one a *Fusicladium* bearing conidia on the lower side of the leaf, the other a *Phyllosticta*.

DESCRIPTION OF THE DISEASE

The first symptom of black-leaf is the development on the lower surface of leaflets and stipules of small, irregular, whitish areas, from 1 to 7 mm. across (Pl. XXVI, A), almost simultaneously with the appearance of slightly pale areas on the upper side. These whitish areas become somewhat larger and then, developing a tan color at the center, darken centrifugally through shades of gray to black. This darkening of the spot is due to the development of a layer of closely packed conidia on the leaf surface. After thus blackening, the center of the spot may grow lighter, changing to gray or olive gray when the conidia have fallen. Spots are limited laterally by the primary and secondary veins, which they seem unable to cross. Longitudinally spots may increase indefinitely, in which case they may be gray at the center, black where fresh conidia stand compactly, and whitish at the advancing margins (Pl. XXVI, A and B). Spots may become confluent near the margins of a leaflet where veins are small. Blackening is not always continuous in an affected spot but may assume an irregularly patchy or reticulate pattern (stipules, Pl. XXVI, A).

Development of this black conidial layer on the lower side of the leaf is not usually followed by collapse of the tissues until after the conidia are

[1] The writer wishes to acknowledge his indebtedness to Professor B. L. Richards, of the Utah Agricultural College, at whose suggestion this work was undertaken, for advice and encouragement throughout the course of this study; also to Dr. J. J. Davis, of the University of Wisconsin, who has aided in the taxonomic study.

shed. The spots then dry to brittleness and may crack out (Pl. XXVI, C). In such late stages a spot may be black above as well as below. In exceptional cases a few conidia are borne on the upper face of a diseased spot. Plate XXVI, D and E, shows the typical appearance of an old spot as seen from the upper and lower sides, respectively.

After conidia-bearing spots have stopped enlarging and most of the conidia are shed, a bluish gray color appears around and sometimes within the conidia-bearing areas. This results from the development, beneath the epidermis, of numerous minute, black, pycnidium-like bodies, more numerous below, but not absent from the upper side of the leaf. Commonly the pycnidial zone is narrow at the margins of old conidial spots, but at times large areas of a leaf may be involved. The pycnidium-like bodies have been observed in material collected in many localities. In no instance have they been observed in a leaflet that had not previously borne conidia. As with the conidial areas, collapse of the tissues usually does not follow promptly the development of pycnidia; the invaded areas are not margined.

The black-leaf disease develops less abundantly and more tardily on petioles and tendrils than on leaflets and stipules, but in the later stages it is not uncommon to find tendrils completely girdled and petioles bearing elongate spots. A spot on a petiole, even if it girdles, does not result in prompt killing of the leaflets; there seems to be no direct interference with the conductive tissues. As a rule production of conidia is reduced, and the production of pycnidia is relatively more abundant than on the laminae.

Black-leaf has rarely been observed in a field before the peas approach full blossom. Developing on the lower leaves at first, spots appear progressively higher on the plant until, when the peas are ready for canning, spots are generally developed even on the uppermost leaves (Pl. XXVI, F).

VARIETAL SUSCEPTIBILITY

No comparative trials of varieties have been made, but the black-leaf disease has been observed on the following varieties:

White (Yellow) Admiral	Green Admiral
Horal	Alaska
Champion of England	Sugar Marrowfat

Of these the White Admiral appears to be the most susceptible. In Utah the disease has been reported only from canning factory districts in which this is the dominant variety grown. In several districts where the disease has been sought in vain, this variety is not grown. In a number of instances fields of Alaska and White Admiral were seen growing side by side; in all such cases the disease, if present, was most abundant in the Admiral. In

Idaho the experience of a prominent seedsman indicates that the White Admiral is more frequently affected than any other variety.

PRESENT DISTRIBUTION

Black-leaf is known at present to occur only in three valleys in northern Utah and in the great seed pea producing area in the Snake River Valley of Idaho, from Idaho Falls to Ashton. In the Cache, Ogden, and Weber valleys of Utah the disease has been observed each year since it was first seen in 1921, varying greatly in severity. During the dry summer of 1924 it was all but absent. In Idaho the writer has seen black-leaf only in the summer of 1925. One of the leading seedsmen, however, described black-leaf in an unmistakable manner as a disease affecting the White Admiral pea in wet seasons in that region for the past ten years.

ECONOMIC IMPORTANCE

Black-leaf is probably not greatly to be feared as a factor limiting pea production in the intermountain region, judging from the behavior of the disease observed during five seasons. Occasional fields have been found, especially in wet seasons, in which more than 50 per cent of the leaf surface has been destroyed by the time the crop has reached the stage for canning. In such cases there has been a marked reduction in yield, but generally the disease causes only moderate foliar destruction at so late a date that losses are negligible. In seed fields, owing to the longer time the crop must remain in the field, the disease may cause somewhat greater reductions of yield, but it has not been looked upon as of serious importance by seedsmen or growers.

MICROSCOPIC STUDIES

Microscopic studies of sections through young spots on leaves collected when symptoms first become discernible reveal the presence of a slender, sparingly branched, septate mycelium growing through the intercellular spaces of the leaf. Hyphae may be seen lying in intimate contact with host cells and extending the length of palisade cells. No haustoria have been detected. There appears to be no immediate reaction of host cells to the presence of these hyphae, but there is a gradual breaking down of the protoplasm, which finally disappears, leaving the cell walls intact. Except in the process of conidia-formation, the mycelium is strictly intercellular. When pycnidia commence to form, the mycelium becomes thicker, more conspicuously septate, and brownish in color. The pycnidia develop from hyphal knots in the intercellular spaces and gradually increase in size, crowding back the empty host cells. Thickened hyphae may fill the intercellular spaces, but as a rule the host cells retain their shape even after they are dead

(Pl. XXVII, A). Early death and collapse of some spots has been observed to inhibit the normal development of conidia and especially of pycnidia.

The apparent inability of the parasite to cross the larger veins of a leaf may be accounted for by its strictly intercellular habit. Above and below the larger veins is a ridge of collenchyma in which intercellular spaces are almost absent. In leaf sections hyphae may be seen advancing far into the available spaces but unable to make their way across such veins. In tendrils and petioles the distribution of intercellular spaces markedly restricts the advance of the fungus. As shown in Plate XXVII, B, the sub-epidermal layers of parenchymatous chlorenchyma in which large intercellular spaces are present are thoroughly invaded, but the more compact interior tissues remain free from invasion. Probably the failure of black-leaf to develop on pea stems is in part associated with the smaller size and extent of intercellular spaces in the sub-epidermal layers as compared with corresponding layers of the petiole.

DEVELOPMENT OF CONIDIA

Shortly after mycelium has appeared in the intercellular spaces of a leaf, hyphae may be seen growing in close contact with the cells of the lower epidermis. From such hyphae branches arise at right angles and grow directly through the inner wall of the epidermis into the epidermal cells. Swelling slightly, they continue to elongate until they push out through the outer wall, penetrating apparently by mechanical means. Protruding from the lower epidermis, these branches then enlarge into conidiophores which are clavate while young and are filled with dense cytoplasm. They are for a time non-septate and uninucleate, the large nucleus remaining near the base within the epidermal cell. A single bud-like projection then appears at the apex and proceeds to enlarge. It may be smoothly ovoid or may be elongate, marked early by a slight constriction at the place where septation may later develop. As the young conidium approaches mature size, the nucleus from the base of the conidiophore passes upwards, divides, and one daughter nucleus passes into the conidium. Septation by constriction then separates the conidium from the conidiophore. The nucleus in the conidium may or may not divide. In case of division the daughter nuclei separate and a septum is formed between them, developing inward from the side walls. The nucleus and cytoplasm remaining in the conidiophore collect in the upper end where they gradually degenerate and disappear. At the same time the upper part of the conidiophore wall becomes thicker and dark brown. By the time the conidium is shed the conidiophore is usually an empty conical structure, broad at the base, the wall of the upper part thickened until it almost closes the lumen, and the extreme tip almost black.

Functioning conidiophores have not been observed to pass through stomata or guard cells. In early stages of the disease hyphal branches may be observed to have grown out through stomatal openings. Such hyphae, as shown by staining reaction, have died after having extended only a short distance (4–10 μ) through the pore, showing as heavily staining clavate hyphae with no indications of the formation of conidia.

The young conidium may early be seen to have a double wall, the inner homogeneous in appearance, staining lightly; the outer dark brown and warted, staining deeply. The outer wall does not become thick, but its warted nature becomes more pronounced at maturity.

Mature conidia (Pl. XXVII, C) are typically ovate to cylindrical, constricted at the septum, rounded at the ends, and flattened at the point of attachment to the conidiophore. The lower cell is characteristically somewhat the larger. Shape varies with compactness of sporulation, spores borne in crowded masses being more slender than the type. The color in mass is dark olivaceous brown to black; under the microscope, a dilute brown.

TAXONOMY

The shape, septation, color, and mode of production of conidia place the fungus in the hyphomycetous genus *Fusicladium* Bonorden. This fungus differs from other species of this genus and bears some resemblance to *Heterosporium* Klotzsch in the roughening of the spore wall, but no spores have been observed with more than one septum. Recently von Höhnel (3) has proposed a revision of the genera *Fusicladium* and *Passalora*, suggesting a number of new genera. Since the advisability of this revision is questioned and since none of the newly defined genera appear to include this fungus, the genus as defined by Bonorden (1) is here accepted.

Only two species of *Fusicladium* are known to have been listed as occurring on peas. Oudemans (6) lists *Fusicladium pisi* Oud. and refers to an earlier paper (4) for the description. In this earlier paper there is no mention of this binomial. Instead, *Fusicladium fagopyri* Oud. is described as occurring on the leaves of peas. Oudemans, in his later work (5), states that he had been in error in listing *F. fagopyri* as occurring on peas. Thus the binomial *Fusicladium pisi* Oud. stands in literature with no description. As for *F. fagopyri*, Oudemans's description does not fit the fungus under present consideration; his spore measurements of 14×9 μ are less than the minimum dimensions of the septate spores, and his measurements of conidiophores are several times longer than the conidiophores of this fungus.

Fusicladium brevipes Ell. and Ev. was described in 1889 (2) from specimens collected in the Sangre de Christo mountains of Colorado, occurring on *Astragalus hypoglottis*. The fungus on peas was tentatively but erroneously considered to be this species and was so reported in the Plant

Disease Reporter (7). Type specimens of this fungus from Colorado have been examined, through the kindness of Dr. Seaver of the New York Botanical Garden, and it appears to be entirely distinct from the fungus on peas. The spores are much longer, frequently biseptate, and have smooth walls. No other species of this genus are known to occur on plants closely related to *Pisum sativum*. The present fungus is therefore designated a new species, described as follows:

Fusicladium pisicola n. sp.

Mycelium intercellular in leaf parenchyma; spots dark gray, olivaceous, or black, immarginate, irregular to oblong, restricted in lateral extent by the larger veins, hypophyllous or rarely amphigenous; conidiophores growing through the epidermis, short, erect, continuous, conical, the walls thickened and dark brown at the truncate apex, 10–24 μ long and 4–6 μ broad above; conidia solitary on tips of the conidiophores, ovate to cylindrical, rounded at ends or truncate at base, generally becoming uniseptate, constricted at the septum, the cells equal or the lower somewhat larger, walls with fine wart-like roughening, brown, 16–32 × 8–14 μ.

On leaflets, stipules, tendrils, and petioles of *Pisum sativum* in Utah and Idaho.

THE PYCNIDIAL FUNGUS

The bodies which develop around and sometimes within the conidial areas may be seen under the microscope to be of two distinct classes: one a pycnidium, the other a sclerotial body. The sclerotial bodies are always in the majority; in fact pycnidia are often very rare. The pycnidia are spherical to ovoid with thin but distinct walls, averaging 60 × 70 μ. At first sub-epidermal, they finally become erumpent. Within the pycnidia, numerous, minute, hyaline, continuous, rodshaped spores are produced from the tips of pointed conidiophores which line the cavity. The spores are variable in size and are often poorly developed, attaining a maximum size of about 1.3 × 5 μ. This fungus is evidently a *Phyllosticta*.

Although several species of *Phyllosticta* have been described on *Pisum sativum* and related hosts, all seem different from this fungus either in size of spores or in the nature of the spot produced. Since, however, its relationship to *Fusicladium pisicola* remains uncertain, no specific name is given at present.

The sclerotial bodies average somewhat larger than the pycnidia, ranging from 75 to 90 μ in diameter. Resembling the pycnidia very closely in shape, they differ in having a slightly thicker wall and in being composed of a mass of interwoven, loosely packed, irregular, hyaline mycelium. No spores of any kind are associated with these bodies during the summer, but some indications suggest that they represent a stage in the development of

perithecia. That the sclerotial bodies are produced by the same fungus as develops the pycnidia is shown conclusively by the occurrence of occasional compound structures surrounded by one continuous wall, one-half being of typical pycnidium formation and the other half having typical sclerotial structure.

No such positive evidence is at hand concerning the relation of these structures to the *Fusicladium*. Microscopic evidence as well as field observations tend to indicate that the pycnidia, sclerotial bodies, and conidia are all associated with the same fungus, but only pure culture methods or inoculation studies can positively determine the connection.

OTHER FUNGI

While other fungi have been observed associated with black-leaf spots, none has been found with any degree of constancy in the earlier stages of development. The only one of these worthy of mention is a species of *Cladosporium* which appears as a common saprophyte on pea vines in the localities studied. This fungus is found sporulating in old conidial spots where it helps to produce the characteristic olivaceous color of old spots. The spores, catenulate, olive green, finely echinulate, pointed at each end, and continuous to biseptate, are readily distinguished from the conidia of *Fusicladium pisicola*. This fungus appears to hasten the collapse of invaded spots and is frequently present when pycnidia and sclerotia are commencing to form, but it probably has no connection with them.

ATTEMPTS TO CULTURE THE PARASITE

During three successive summers attempts were made to grow the *Fusicladium* in culture but without success. Dilutions of conidia were made in various ways. Spores were sown on and in various agars, and single-spore transfers were made to a variety of media. Spores from different localities and from young and old spots were tried, some fresh from the field and some that had been in storage for various lengths of time. The media used include water agar, prune agar, raisin agar, potato agar (with and without dextrose), nutrient agar (with and without dextrose), oatmeal agar, pea vine agar, cornmeal, steamed pea vines, pea vine decoction, and raisin decoction.

Conidia germinate freely on most of these media, producing germ tubes which reach their maximum development within 48 hours, seldom exceeding 140 μ in length. Single-spore transfers have been observed for as long as two weeks without further growth being detected. Besides dilution cultures, direct leaf-fragment platings were made in various ways, not only from conidial spots but from pycnidial areas as well, but with no better results. In all these attempts, *Cladosporium* was the fungus most frequently

obtained, but in no case did a spore marked as typical of *Fusicladium pisicola* produce a culture of *Cladosporium*.

FACTORS INFLUENCING GERMINATION

Conidia of *Fusicladium pisicola* were found to germinate only rarely in distilled water and poorly on water agar, a small percentage of spores giving rise to tubes apparently lacking in vigor. In prune and raisin decoctions much higher percentages of germination and stronger germ tubes were observed.

Temperature was observed to have a marked influence upon both percentage of spores germinating and type of germ tube produced. Two suspensions of conidia in prune decoction were divided into three lots, and one lot of each was held at temperatures of 7°, 17°, and 27° C. After 18 hours the percentages of spores germinated were as follows:

	7° C.	17° C.	27° C.
Sample A	50 per cent	66 per cent	25 per cent
Sample B	24 per cent	40 per cent	28 per cent

Not only did a higher percentage of spores germinate at 17° C. than at the other temperatures but the germ tubes attained greater maximum length. At 7° C. germ tubes were short and thick with a strong tendency to branch, while at the highest temperature germ tubes were very slender and with no branching. Those produced at 17° C. showed an intermediate thickness and but little branching.

Spores were applied dry, in drops of water, and in drops of dilute prune decoction, to the surface of green pea leaves under sterile conditions in petri dishes kept moist with wet filter paper. In all cases germination was rapid and vigorous. Leaf fragments bearing these germinating conidia were examined after being cleared in absolute alcohol-glacial acetic acid mixture but no tendency towards penetration was observed.

The length of time that spores have been found to remain germinable is variable. Some collections have shown prompt decline in germinability, while one collection germinated well in April, after having been in the herbarium nine months.

INOCULATION EXPERIMENTS

A number of inoculation experiments have failed to produce infection. Conidia have been applied to leaves of the White Admiral pea in various stages of development under a variety of conditions: applied dry and suspended in water; with and without first rubbing the bloom from the leaf surface; with and without injury to the epidermis. Several devices have

been used to maintain humidity. Introduction of conidia-bearing vines into plots of healthy peas has resulted in no infection.

DISCUSSION

The occurrence of a disease of some importance in a seed growing area from which great quantities of seed peas are shipped to the major pea canning regions of the country may be of some concern to users of this seed in districts where the disease has not appeared. While it is not yet possible to say with certainty whether this disease may be disseminated with pea seed, this seems unlikely. Black-leaf is not known to affect either the seeds or the pods. Any transfer of the fungus would therefore be only as spores adhering to the seeds. The chance of conidia borne thus on the seed being able to survive and to establish infection seems very small.

Until more is known of the life history of the parasite and of its relation to environment, it is impossible to say just what has limited the disease to the intermountain region. That black-leaf does not occur at present in Wisconsin seems certain: the writer, in conducting an intensive survey of diseases of canning peas in that state during 1924 and 1925, has been constantly on the alert for it. If, as pointed out earlier, black-leaf has been present in the seed districts of Idaho for a decade, it seems certain, in view of the great amount of seed annually shipped to Wisconsin, that either the disease is not seed-borne or that some factors inhibit its development in Wisconsin.

SUMMARY

1. Black-leaf of peas is a newly described disease characterized by dark olivaceous gray to black elongate spots on the lower side of leaves and on petioles and tendrils.

2. This disease is now known to occur in three valleys in northern Utah where peas are grown for canning, and in the great seed pea-producing area of the Snake River Valley, Idaho.

3. While black-leaf may cause considerable losses under favorable conditions, it has not been of great economic importance under usual conditions.

4. The earliest symptom of this disease is due to the development of conidia of a fungus described as *Fusicladium pisicola* n. sp. Associated with the older spots are pycnidia of Phyllosticta type and sclerotia of similar external appearance. The development of the fungus in the leaf is briefly traced.

5. The conidia germinate freely under a variety of conditions but do not continue their growth on the artificial media tried. The fungus has not been grown in culture. Preliminary inoculation studies have not established infection.

6. Positive evidence is lacking as to the possibility of black-leaf being introduced into new localities with seed from infested fields, and available information suggests that the chance of such introduction is slight.

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EXPLANATION OF PLATES

PLATE XXVI

A. Lower surface of leaf bearing numerous black-leaf spots in early and medium stages of development. The pale spot at 1 represents the earliest symptom of this disease. At 2 is a spot in which the central blackening is becoming apparent. ×2/3.

B. Group of spots at base of leaflet, showing lateral limitation by the larger veins and the terminal black bands where conidia stand compactly, marking the ends of spots that are elongating. ×1 1/3.

C. Late stages of the disease, showing cracking out of old diseased areas. The spots have become confluent near the leaf margin but are still separated by the larger veins towards the center. ×2/3.

D. Upper side of leaflet, showing typical appearance of a black-leaf spot in its later stages. Compare with E. ×1 1/3.

E. Lower side of leaflet, showing same spot as in D. Pycnidial bodies occur in a narrow band at margin of spot. ×1 1/3.

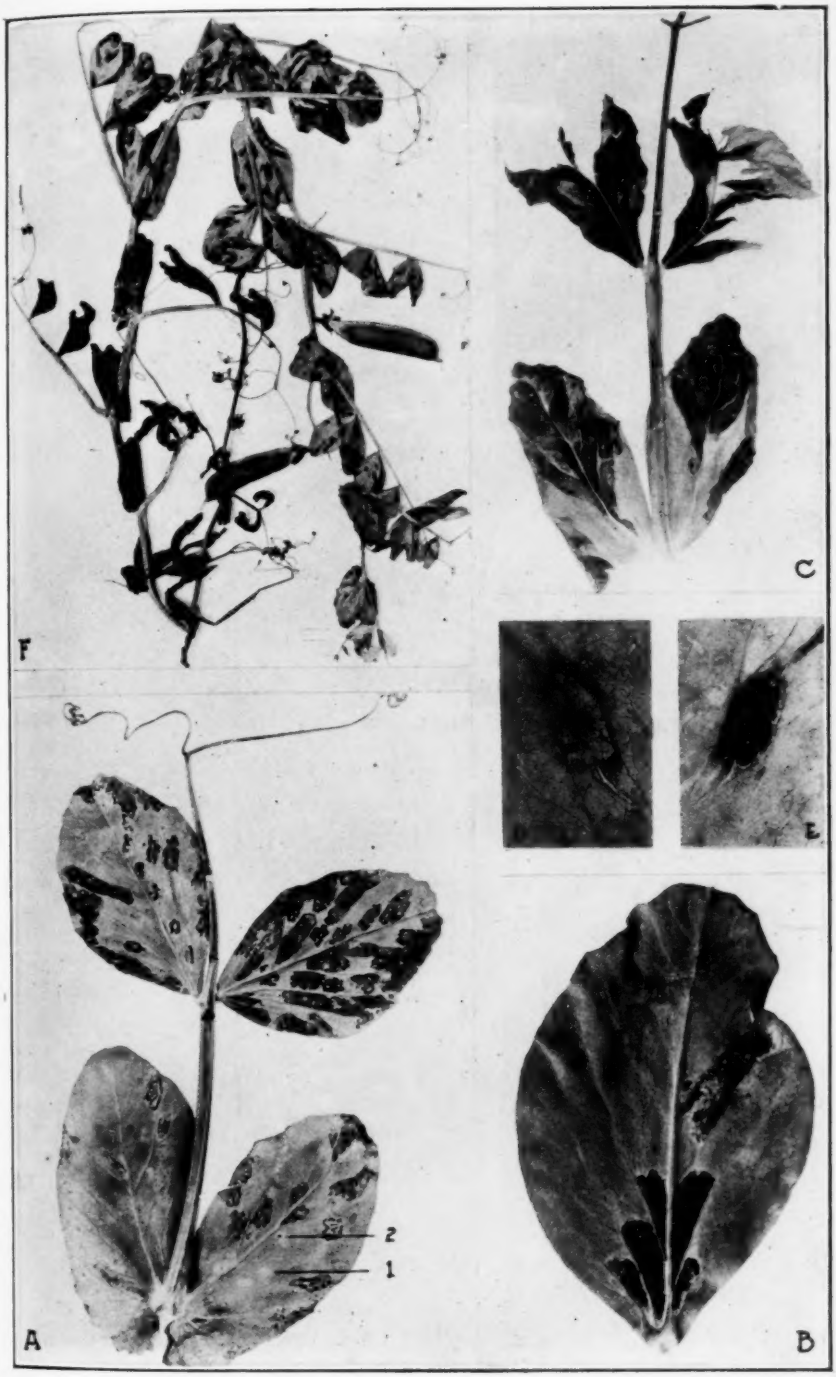
F. A plant at canning age showing heavy infection in late stages. The uppermost leaves are spotted but the pods are disease-free. The lower leaves have been killed. ×1/5.

PLATE XXVII

A. Traansverse section through pycnidial area at margin of a conidial spot, showing at the right a sclerotial body and at the left a pycnidium filled with minute spores. The thickened mycelium is strictly intercellular, leaving the empty palisade cells (above) intact. ×500.

B. Portion of transverse section of petiole showing the limitation of the mycelium to the outer region in which large intercellular spaces are continuous. At the left the epidermis is roughened by the persistence of old conidiophores. Two sclerotial bodies are shown. ×150.

C. Conidial layer of *Fusicladium pisicola* at lower side of leaf, showing septate conidia borne apically on short conidiophores arising through the epidermis. × 500.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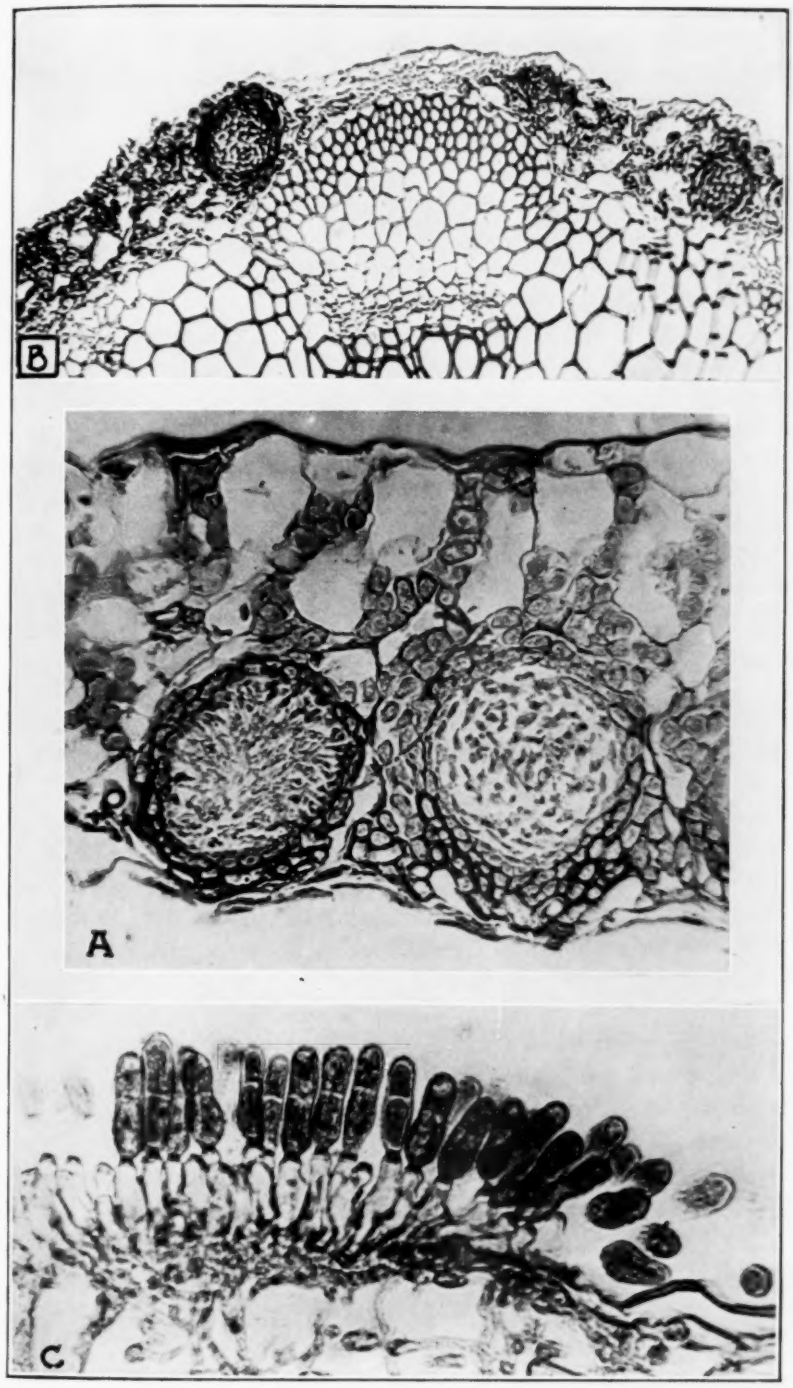
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C

PRELIMINARY REPORT OF SOME OBSERVATIONS ON ASCOSPORE DISCHARGE AND DISPERSAL OF CONIDIA OF VENTURIA INAEQUALIS (COOKE) WINTER

J. E. Howitt and W. G. Evans

The general life history of *Venturia inaequalis* (Cooke) Winter has been known for many years. The inoculum for primary infection in the spring of the year comes chiefly in the form of ascospores from the fallen leaves. Secondary cycles are initiated by conidia produced in the developing scab spots. Spore discharge of the apple scab fungus has received considerable attention from plant pathologists. Wallace (7), Childs (2), Curtis (3, 4), Keitt and Jones (5), Schneiderhan (6) and Adams (1) have all published important contributions to our knowledge on this subject. There are, however, many problems of importance in regard to the details of the life history which have not been completely solved. Some of these are: the relationship of temperature and precipitation to ascospore development and discharge; the length of period of ascospore vitality; and the dissemination of conidia through the orchard.

With the hope of throwing more light on these and other problems, observations have been made and recorded during the past six years. Unfortunately the records for 1921 have been mislaid and consequently cannot be included in this preliminary report. These observations have been made each year in a neglected orchard near the Ontario Agricultural College and supplemented by observations made in other neglected orchards in the neighborhood. Records have been kept of the date of first ascospore discharge for each year and the date of free ascospore discharge for each year. In addition the development of the apple buds at the time of ascospore discharge also was recorded. These observations were supplemented by germination tests and field experiments.

The time of first ascospore discharge and free ascospore discharge was determined by gathering apple leaves at different dates during the spring and examining the perithecia to ascertain the stage of development of the asci. The date of first ascospore discharge was judged by the first appearance of empty asci in the perithecia. The date of free ascospore discharge was considered to be the date when fifty per cent or more of the asci were found to have discharged their spores. The dates recorded therefore are necessarily but approximate. They should, however, suffice for a basis of comparison.

THE RELATIONSHIP OF TEMPERATURE AND PRECIPITATION TO ASCOSPORE DEVELOPMENT AND DISCHARGE

Such a relationship has long been recognized by all who have worked with apple scab. The consensus of opinion appears to have been that the amount of precipitation during April and May was the important factor influencing the development and discharge of the ascospores. The figures given in the table below, while not absolutely conclusive, strongly indicate that the temperature during the winter months may be a factor, the importance of which up to the present time has not been fully recognized.

Table 1 records figures for the years 1919, 1920, 1922, 1923 and 1924 only. The records for 1921 have been mislaid, and the records for 1925, not having been made in the neighborhood of Guelph, are not comparable. The available soil temperatures at the depth of one inch have also been included to show that the varying snowfalls of the different seasons did not appear to alter the ratio of the average mean temperature for the winter months of the years recorded.

A study of the figures in the table brings out the following points.

First, that apparently the average mean temperature of the months of January, February and March has more influence upon ascospore development and primary discharge of ascospores in the spring than has the amount of precipitation from April 1 to primary ascospore discharge. These figures do not prove this conclusively but they at least strongly suggest it.

Second, that the average mean temperature for January, February and March is more closely related to ascospore development and discharge than is the mean temperature of the days in April and May preceding primary ascospore discharge.

When the average mean temperatures for the preceding six months, namely, October, November, December, January, February and March, of the various years recorded were compared with the dates of primary ascospore discharge and free ascospore discharge, a correlation was found which, although not complete, was sufficiently marked to indicate that the temperatures of the preceding six months, as well as those of January, February and March only, may have an influence upon ascospore development and discharge.

LENGTH OF PERIOD OF ASCOSPORE VITALITY

The length of time that ascospores capable of germination remain in the orchard is also a question of some importance. Observations throwing some light on this point were made in 1925. The first mature ascospores were found on April 23. Each week from this date until August 8, viable ascospores were found. The vitality of the ascospores was determined by germination tests made by placing the ascospores in water on slides. These tests were made at ordinary room temperatures. It is interesting to note

TABLE 1.—*Precipitation and temperature in relation to ascospore development and discharge in Venturia inaequalis*

Year	1919	1920	1922	1923	1924
Date of first observed ascospore discharge	April 28	May 17		May 18	May 9
Date of free ascospore discharge	May 6	May 31	May 10	May 26	May 17
No. days from April 1 to free ascospore discharge	36	61	40	56	47
No. days from first observed ascospore discharge to free ascospore discharge	8	14		8	8
No. days of precipitation from April 1 to free ascospore discharge	15	26	8	22	20
Amount of precipitation in inches from April 1 to first ascospore discharge	2.58	3.69		5.61	4.64
Amount of precipitation in inches from first ascospore discharge to free ascospore discharge	3.04	.65		1.43	3.09
Mean temperature, January	26.3	9.59	18.97	18.8	19.4
Mean temperature, February	25.3	14.55	23.18	14.1	16.33
Mean temperature, March	24	30.67	32.08	24.28	27.9
Average mean temperature for January, February, and March	28.53	18.27	28.07	19.06	21.21
Average mean soil temperature at depth of 1 inch for January, February, and March	30.23	27.39	29.65		
Mean temperature from April 1 to first ascospore discharge	39.2	42.5		43.5	42.5
Mean temperature from April 1 to free ascospore discharge	40.4	45.36		43.5	44.5
Mean temperature from first ascospore discharge to free ascospore discharge	44.8	60.1		53.3	50.2

that apple leaves collected on August 7 contained perithecia filled with immature asci in which the protoplasm was still undifferentiated, and that apple leaves which had been kept in a cage outside under some trees, where they were exposed to temperature changes but not to soaking by rain, were found to contain asci and ascospores when examined in December.

DISSEMINATION OF CONIDIA THROUGH THE ORCHARD

The question as to whether conidia are blown by the wind from tree to tree in an orchard is one of some practical importance but one upon which there appears to be little evidence based on experiments. Some pathologists evidently assume that such dissemination does take place, while others appear to think that there is little danger of conidia blowing from tree to tree. To secure evidence bearing on this question, experiments were conducted in 1925.

On June 5, a number of stakes were placed at an angle of 45° to the ground around an isolated tree in an orchard. These stakes were located at distances of from 3 to 40 feet from the tree affected with scab. Three to five microscopic slides, lightly covered with vaseline, were fastened to each of these stakes. Similar slides were also fastened to nearby fence posts. On June 30, a few conidia were found on each slide. These slides were on stakes placed 3, 6, 10 and 15 feet from the tree trunk. On July 2, a few conidia were found at 3 and 10 feet from the trunk. On July 8, conidia were found west and southwest of the tree on slides attached to stakes at distances of 5, 12, 15 and 25 feet from the trunk, and on slides on two fence posts 50 feet from the tree trunk. At this time scab was developing rapidly on the leaves of the tree. On July 16, another set of similar slides was placed around the tree. On July 20, slides at 10, 20, 30 and 40 feet north of the tree showed conidia; and on July 28, slides on stakes located 5, 10, 20, 30, 40 and 50 feet west of the tree showed conidia. Conidia were also found on slides fastened to fence posts 75 feet from the tree. This tree was nearer the slides than any of the other apple trees. The conidia collected on these slides were not distinguishable from the conidia scraped from the scab spots on the leaves. On slides placed in a field at some distance from the orchard, no spores resembling the conidia of *Venturia* were found.

SUMMARY

1. The mean temperature of the winter months, especially of January, February and March, has a marked influence upon ascospore development and primary discharge in the spring in *Venturia inequalis*.

2. Ascospores, in Ontario, may retain their vitality at least until the eighth of August, and probably until a much later date.

3. Conidia may be freely scattered by wind from tree to tree in an orchard.

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SEED TREATMENT EXPERIMENTS FOR CONTROLLING STRIPE DISEASE OF BARLEY[1]

R. W. Leukel, James G. Dickson, and A. G. Johnson

INTRODUCTION

During the past fifteen years, various workers have conducted numerous experiments to find a satisfactory seed treatment for the control of stripe disease of barley caused by *Helminthosporium gramineum* Rabh. Most of this work was done in Europe, where stripe disease appears to be most serious. The earlier experiments were confined mostly to the use of hot water, mercuric chloride, copper sulphate and formaldehyde, but organic mercury and other compounds were tried as early as 1914.

Mortensen (17) in 1909 tried the hot-water treatment. Störmer (34) in 1912 also tried hot water as well as copper sulphate and formaldehyde. Appel and Riehm (4) in 1913 tried hot water with varying results. Johnson (11) in 1914 reported good results from the use of hot water and formaldehyde. Spiekermann (33) in 1914 failed to control stripe by the use of hot water and formaldehyde. Riehm (27) in 1914 and again (28) in 1915 reported excellent results from the use of chlorophenol mercury. Johnson (12) in 1916 used formaldehyde and copper sulphate with good results. Kiessling (14) in 1916 reported partial control with Uspulun but found considerable variation in its effectiveness on different varieties.

Müller and Molz (18) in 1918 experimented with a large number of substances not commonly used for seed treatments, their aim being to eliminate bird and rodent depredations, as well as stripe disease. Lind and Ravn (16) in 1918 reported the results of eight years' work with various seed treatments and recommended copper sulphate, mercuric chloride or formaldehyde for severely infected seed, and hot water for slightly infected seed. Experiments in Scotland (1) in 1919 included these same treatments. Köck (15) in 1919 recommended formaldehyde and Uspulun. Müller, Molz and Shröder (19) in 1920, reporting the results of three years' experiments, stated that Germisan controlled stripe disease perfectly, while Uspulun and Corbin reduced the percentage of infection from 30 per cent to 4.7 and 6.3 per cent, respectively.

Riehm (29) in 1921 reported good results with Uspulun and Fusafine. Nolte and Gehring (22) in 1921 reported excellent results from the use of

[1] Investigations conducted cooperatively between the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

formaldehyde, Uspulun and Fusafine. Müller and Molz (20) in 1921 again reported complete control of stripe disease with Germisan, and fair control with Uspulun. Fraser (8) in 1921 reported satisfactory control with hot water and formaldehyde, reducing the percentage of stripe disease from about 60 per cent to 11 per cent with the former, and to 5 per cent with the latter. Verhoeven (35) in 1921 secured approximate control by spraying different lots of seed with solutions of copper sulphate, Germisan 14 B, and Uspulun. He tried soaking the seed in Uspulun solution for 1½ hours and then in hot water (52° C.) for 10 minutes. He also used a hot Uspulun solution after previously soaking the seed in cold water. In both cases, he reported excellent stripe control.

Riehm (30) in 1921 recommended formaldehyde and Uspulun for perfect control of stripe, and stated that mercuric chloride, copper sulphate and Fusafine greatly reduced the amount of infection. Schaffnit (32) in 1922 reported complete control of stripe disease with Germisan, and approximate control with Uspulun. He found these two the only effective treatments among a considerable number tried. In 1923, van Poeteren (25) recommended Uspulun, Germisan and copper sulphate. Ausborn (5) in 1923 recommended Germisan, Uspulun and hot water. Verhoeven (36) in 1923 stated Germisan produced the best results in stripe control, while Uspulun was fairly good. Dreger (6) in 1923 secured perfect control by using Uspulun after hot water. Remy and Vasters (26) in 1923, after reviewing the work done on seed treatment from 1913 to 1920, recommended chlorophenol mercury for perfect control of stripe disease. Müller, Molz and Müller (21) in 1923 again recommended Germisan and Uspulun.

Plaut (23) in 1924 reported favorable results with Germisan, Uspulun and Tillantin "C," and again (24) in 1925 with the first two. He also tried many other treatments, including hot solutions of some chemicals. Adams (3) in 1924 advised treatment with Semesan. Ferdinandsen (7) in 1924 pronounced Germisan the best preparation yet discovered for the control of stripe disease.

Gram (9) in 1924 recommended Germisan as being superior to Uspulun and copper sulphate for stripe control. In another report (2) in 1924 Germisan, Tillantin "C" and "Hohenheimer Beize" are recommended. Johnson, Leukel and Dickson (13) in 1924 reported perfect control of stripe disease with solutions of Semesan, Chlorophol, Germisan, Corona 610 and Corona 620, without seed injury. Gram (10), in 1925, after extensive experiments covering seven years, recommended Germisan above all other treatments, and next to that, Tillantin "C." Rodenhiser and Stakman (31) in 1925 reported satisfactory control of stripe disease with hot solutions (52° C.) of Germisan, Uspulun and Semesan. Germisan solution at room temperature also was satisfactory.

The foregoing summary of the more pertinent literature on seed treatments for the control of stripe disease of barley shows considerable variation in the results obtained by different workers using seed-treatment materials of the same name. Following are some of the probable reasons for these apparent discrepancies.

1. The variety of seed used. Some varieties respond to treatment more readily than others (4, 15).
2. The severity of the seed infection.
3. Differences in the methods and the details of applying the treatments, as, the temperature of the solution, the amount of stirring and mixing, the degree of contact between seed and solution, the duration of the treatment, the method of drying the seed, and the number of times the solution was used without renewal.
4. Variation in the chemical composition of compounds of the same name used at different times or by different workers.
5. Variation in the conditions obtaining at the time of sowing the seed, such as soil temperature, soil moisture and soil reaction.
6. Lack of adequate planting replications.
7. A difference of opinion as to what constitutes "satisfactory control."

The inoculum furnished by a small percentage of stripe disease in a crop may cause considerable seed infection. It seems that unless seed treatment is to be practised every year, a satisfactory treatment for stripe disease should eliminate the disease completely without seed injury.

In earlier experiments conducted by the writers, hot water, formaldehyde and copper sulphate were not found entirely satisfactory, chiefly because of seed injury and lack of consistent effectiveness.

In the fall of 1922, the writers started experiments with a number of the most promising, new seed-treatment materials. A summary of the earlier results has been published (13). The manufacture of some of these compounds first used has been discontinued, while other compounds have been discarded because of ineffectiveness. On the other hand, new seed-treatment compounds appeared upon the market too late to be included in the experiments here described.

MATERIALS

In the spring of 1925, rather extensive seed-treatment experiments were undertaken, especially with liquid treatments. Only those compounds that seemed most promising and were then available were used. All compounds employed were of recent manufacture. The following compounds were used:

A. For liquid treatments.

1. Uspulun, a hydroxymercurichlorophenol sulphate preparation, produced by the Bayer Company, Inc., New York City.
2. Bayer Compound, a hydroxymercurinitrophenol sulphate compound, also produced by the Bayer Co., Inc., New York City.
3. Germisan, a mercury cresol-sodium cyanide compound, manufactured by the Saccharin-Fabrik Aktiengesellschaft, vorm Fahlberg List and Company, Magdeburg, Germany.
4. S. F. A. No. 125, and
5. S. F. A. No. 175; the chemical composition not known to the writers; both manufactured by the Saccharin-Fabrik Aktiengesellschaft, vorm Fahlberg List and Company, Magdeburg, Südost, Germany.
6. Semesan, a chlorophenol mercury compound made by E. I. du Pont de Nemours and Company, Inc., Wilmington, Del.
7. Corona No. 620, a mercurated ortho-nitro-phenol and
8. Corona No. 640, a mercurated acetaldehyde; manufactured by the Corona Chemical Division of the Pittsburgh Plate Glass Co., Milwaukee, Wis.
9. Tillantin "C," a colloidal copper and organic arsenic compound made in Germany and sold in this country by H. A. Metz and Company, Inc., 122 Hudson St., New York City.

B. For dust treatments.

10. Uspulun-Bolus, similar in composition to Uspulun, but in the form of a dust, and
11. Bayer No. 3, the same as Bayer Compound, but to be used in dust form; manufactured by the Bayer Company, Inc., New York City.
12. S. F. A. No. 225, manufactured by the makers of Germisan.
13. Semesan, the same as No. 6, used as a dust.
14. Semesan Jr., made by the manufacturers of Semesan.
15. Copper Stearate, made by the manufacturers of Corona No. 620.

The seed used in the 1925 experiments was Oderbrucker barley, Wisconsin Pedigree No. 6 (C. I. No. 1146), secured from the Agronomy Department of the Wisconsin Agricultural Experiment Station and grown on West Hill Experiment Farm near Madison, Wis. Three lots of this seed from the 1922, 1923, and the 1924 crops, respectively, were used for the liquid treatments. The seed had been cleaned by means of a fanning mill before it was received.

All the seed treatments were applied at Arlington Experiment Farm, Va., by one of the writers, in February, 1925.

METHODS AND RESULTS

Liquid Treatments. For the liquid treatments, 1, 0.5, and 0.25 per cent water solutions of each compound were carefully made up in glazed, earthenware jars. The temperature of the solutions was kept at about 18° C. throughout the treatments. The seed was placed in properly labeled, clean, cheesecloth bags, 100 to 200 gms. in each, and immersed in the solutions for the given lengths of time. After being allowed to drain a few minutes in the bags, the seed was spread out on paper towels to dry. The drying was hastened by the use of electric fans. When thoroughly dry, the seed was packeted in envelopes, 16½ gms. in each, for subsequent sowings in rod-rows at the rate of one gram per linear foot of row.

On account of the shortage of the stripe-infected seed, a closely similar barley, Wisconsin Pedigree No. 9, 1924 crop, was used to test the effects of the treatments on germination. A portion of this seed was treated similarly and 500 kernels from each lot of treated seed and eight lots of 500 kernels each of untreated seed were sown in the field at Arlington Experiment Farm, Va., April 13, 1925. The germination counts, made May 2, are shown in table 1.

TABLE 1.—*Germination percentages of Wisconsin Pedigree No. 9 barley, grown in 1924, untreated or treated with different seed disinfectants and sown in the field in paired rows, 250 seeds per row, replicated twice, at Arlington Experiment Farm, April 13; data recorded May 2, 1925*

Seed disinfectants	Germination percentages of seed						
	Soaked 1 hour in water solutions of:			Soaked 2 hours in water solutions of:			Untreated
	1 per cent	0.5 per cent	0.25 per cent	1 per cent	0.5 per cent	0.25 per cent	
Uspulun	92	94	92	89	91	91	77
Bayer Compound	82	90	91	81	86	86	79
Germisan	78	82	85	73	85	83	79
S.F.A. No. 125	80	86	77	79	91	94	82
S.F.A. No. 175	83	91	91	68	84	90	83
Semesan	91	90	86	92	92	86	81
Corona No. 620	55	67	75	40	57	70	77
Tillantin "C"	73	79	79	50	78	76	77

The field sowings of the treated and the untreated stripe-infected seed were made in three essentially parallel series, as follows:

Series I at Arlington Farm, Va., March 16, 1925, Series II and III at Madison, Wis., April 11 and May 9, 1925, respectively.

TABLE 2.—*Summary of data on stripe-disease control in Wisconsin Pedigree No. 6 barley, seed untreated or treated with different seed disinfectants at Arlington Experiment Farm, Va., and sown as follows:*

Series I at Arlington Farm, Va., March 16, 1925. Six replications.
Series II at Madison, Wis., April 11, 1925. Five replications.
Series III at Madison, Wis., May 9, 1925. Four replications.

Seed disinfectants	Seed from crop of	Total number of stripe-infected plants from seed soaked for one hour in a water solution of											
		1 per cent				0.5 per cent				0.25 per cent			
		Series				Series				Series			
		I	II	III	Total	I	II	III	Total	I	II	III	Total
Uspulun	1922	0	1	1	2	0	2	0	2	2	2	1	5
	1923	0	0	....	0	0	0	....	0	2	4	....	6
	1924	0	0	0	0	4	0	0	4	3	2	0	5
	Total	0	1	1	2	4	2	0	6	7	8	1	16
Bayer Compound	1922	0	0	0	0	0	1	1	2	1	0	2	3
	1923	0	0	....	0	0	1	....	1	0	4	....	4
	1924	4	1	0	5	8	1	0	9	15	6	4	25
	Total	4	1	0	5	8	3	1	12	16	10	6	32
Germisan	1922	0	0	0	0	0	0	0	0	0	0	0	0
	1923	0	0	....	0	0	0	....	0	0	0	....	0
	1924	0	0	0	0	0	0	0	0	0	0	0	0
	Total	0	0	0	0	0	0	0	0	0	0	0	0
S.F.A. No. 125	1922	0	0	0	0	2	3	0	5	16	9	1	26
	1923	0	2	....	2	2	2	....	4	5	5	....	10
	1924	7	4	0	11	14	11	0	25	27	23	3	53
	Total	7	6	0	13	18	16	0	34	48	37	4	89
S.F.A. No. 175	1922	0	1	0	1	0	2	0	2	20	4	0	24
	1923	1	0	....	1	0	1	....	1	5	7	....	12
	1924	1	2	0	3	7	2	0	9	24	14	1	39
	Total	2	3	0	5	7	5	0	12	49	25	1	75
Semesan	1922	1	1	1	3	7	2	0	9	14	9	3	26
	1923	0	1	....	1	0	0	....	0	6	1	....	7
	1924	4	3	0	7	9	1	2	12	21	8	1	30
	Total	5	5	1	11	16	3	2	21	41	18	4	63
Corona No. 640	1922	98	47	13	158	128	76	22	226	150	76	21	247
	1923	29	19	....	48	50	33	....	83	71	41	....	112
	1923	89	41	16	146	99	62	16	177	107	76	21	204
	Total	216	107	29	352	277	171	38	486	328	193	42	563
Corona No. 620	1922	1	0	0	1	4	0	0	4	2	1	0	3
	1923	0	0	....	0	1	0	....	1	0	0	....	0
	1924	0	0	0	0	4	1	0	5	5	0	0	5
	Total	1	0	0	1	9	1	0	10	7	1	0	8
Tillantin "C"[a]	1922	1	0	0	1	0	0	0	0	0	1	0	1
	1923	0	0	....	0	0	0	....	0	1	0	....	1
	1924	.....	0	0	0	.....	0	0	0	.....	0	0	0
	Total	1	0	0	1	0	0	0	0	1	1	0	2

[a] Only three replications in Series I.

Total number of stripe-infected plants from seed soaked for two hours in a water solution of												Number of stripe-infected plants from untreated seed				
1 per cent				0.5 per cent				0.25 per cent								
Series				Series				Series				Series				
I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total	Average per row
0	0	0	0	1	1	0	2	0	3	0	3	139	96	20	255	21
0	0	....	0	0	0	....	0	0	1	....	1	117	77	....	194	32
0	0	1	1	3	0	2	5	1	1	1	3	127	95	24	246	21
0	0	1	1	4	1	2	7	1	5	1	7	383	268	44	695	....
0	0	0	0	0	0	0	0	0	3	1	4	.....	97	37	134	17
0	0	....	0	0	1	....	1	1	0	....	1	.....	69	....	69	17
3	1	2	6	4	3	0	7	13	2	2	17	.....	103	22	125	16
3	1	2	6	4	4	0	8	14	5	3	22	.....	269	59	328	....
0	0	0	0	0	0	0	0	0	0	0	0	166	107	33	306	26
0	0	....	0	0	0	....	0	0	0	....	0	130	55	....	185	31
0	0	0	0	0	0	0	0	0	0	0	0	119	98	22	239	20
0	0	0	0	0	0	0	0	0	0	0	0	415	260	55	730	....
2	0	0	2	6	1	0	7	11	6	2	19	.....	108	27	135	17
0	0	....	0	1	1	....	2	3	2	....	5	.....	75	....	75	20
0	0	0	0	9	3	0	12	19	7	2	28	.....	77	18	95	12
2	0	0	2	16	5	0	21	33	15	4	52	.....	260	45	305	....
0	0	0	0	0	0	0	0	3	1	1	5	142	93	24	259	22
0	0	....	0	0	0	....	0	4	1	....	5	123	55	....	178	29
2	0	0	2	3	5	0	8	13	5	3	21	188	89	22	299	25
2	0	0	2	3	5	0	8	20	7	4	31	453	237	46	736	....
0	1	0	1	5	0	2	7	15	2	4	21	.....	97	32	129	16
0	0	....	0	0	0	....	0	1	2	....	3	.....	49	....	49	12
1	0	0	1	4	5	1	10	14	0	6	20	.....	94	10	104	13
1	1	0	2	9	5	3	17	30	4	10	44	.....	240	42	282	....
83	54	18	157	150	67	19	236	198	84	27	309	212	87	40	339	27
27	10	....	37	55	27	....	82	83	33	....	116	109	49	....	158	26
84	37	10	111	96	68	13	177	114	64	13	201	149	97	29	275	23
276	101	28	305	301	162	32	495	395	181	40	626	470	233	69	772	....
0	1	0	1	2	2	0	4	2	1	0	3	.....	122	31	153	19
0	0	....	0	1	0	....	1	0	0	....	0	.....	56	....	56	14
0	0	0	0	1	0	2	3	1	1	0	2	.....	93	17	110	14
0	1	0	1	4	2	2	8	3	2	0	5	.....	271	48	319	....
0	0	0	0	0	0	0	0	0	0	0	0	230	126	32	388	32
0	0	....	0	0	0	....	0	1	0	....	1	108	56	....	164	29
....	0	0	0	.....	0	0	0	.....	1	0	1	.....	100	26	126	16
0	0	0	0	0	0	0	0	1	1	0	2	338	282	58	678	....

Unfortunately, the limited amount of 1923 seed available made it necessary to sow three replications less of this seed lot than of the 1922 and 1924 seed lots of which six replications were sown for each treatment, with the exception of Tillantin "C." A slight shortage of 1924 seed made it necessary to omit the treatment of this seed lot with Tillantin "C," Series I.

Infection counts on the first, second and third series were made May 20–22, July 2–6 and July 15–17, respectively, the number of diseased plants per row being recorded. Representative rows from each treatment and from the controls were pulled and the plants counted so that the number of striped plants per row could be expressed in terms of approximate percentages.

The results showing the degree of stripe control by each of the liquid treatments in all three series are summarized in table 2.

Yields were obtained from certain representative rows in the second series when the grain had ripened. For this purpose rows were selected in which the stripe control was best, together with nearby control rows. These rows were carefully harvested and threshed separately. The threshing was done at the West Hill Experiment Farm, Madison, Wis., with the rod-row thresher of the Agronomy Department, Wisconsin Agricultural Experiment Station. Immediately after threshing, the yield from each row threshed was cleaned in a small fanning mill. The cleaned grain was then carefully weighed. The yields are given in table 3.

Dust Treatments. Preliminary experiments with dust treatments including copper carbonate, Seed-o-san, Corona 640, Napthal dust and a mixture of Semesan and Lux failed to disclose any that satisfactorily eliminated stripe disease.

Another series of dust treatment experiments of limited scope was carried out at Arlington Experiment Farm, Va., in the spring of 1925. Seed from the crops of 1922 and 1923 was used. The dusts were applied by placing sixty grams of the infected seed in a large-mouthed bottle and adding a heaping teaspoonful of the dust to be tested. The bottle was corked and shaken thoroughly until the seeds were coated as much as possible. The excess dust was removed by pouring the seed on a coarse sieve. A different bottle was used for each treatment. The seed from each treatment was sown at Arlington Experiment Farm, Va., March 21, 1925, in paired rod-rows replicated twice. Untreated controls were sown at the same time. The same rate of seeding was used as in the case of the liquid treatments, and the germination percentages of the treated and the untreated seed also were determined in the same manner. Infection counts were made May 27. The germination and infection data are shown in table 4.

TABLE 3.—*Rod-row yields of threshed barley grown from treated seed and from untreated seed in adjacent rod-rows, the average yield per row, and the average approximate percentage of striped plants in each row.*

Replication number	Yields in grams from seed treated with compounds indicated or untreated										
	Uspulun[a]	Untreated	Bayer Comp.[a]	Untreated	Germisan[b]	Untreated	Semesan[a]	Untreated	Corona 620[b]	Untreated	Tillantin "C"[b]	Untreated
1	662	524	590	484	420	484	330	290	350	282	326	282
2	662	520	550	464	420	464	310	260	340	304	310	304
3	612	412	374	390	416	390	500	360	458	286	418	286
4	560	454	420	462	464	462	454	362	442	400	422	400
5	560	508	652	490	632	444	470	444	512	532	600	532
6	494	490	624	508	622	566	452	404	540	472	636	472
7	680	670	544	574	503	600	552	540	490	374	436	374
8	660	574	508	670	690	654	590	640	434	324	514	324
Total	4890	4152	4262	4042	4167	4064	3658	3300	3566	2974	3662	2974
Average	611	519	533	505	521	508	457	413	446	372	458	372
Average percentage stripe	t[c]	8	t	8	0	7	t	7	0	9	t	9

[a] Seed soaked one hour in 0.5 per cent solution.

[b] Seed soaked one hour in 0.25 per cent solution.

[c] t = trace.

TABLE 4.—*Stripe disease control in Wisconsin Pedigree No. 6 barley, seed untreated, or treated with different dust disinfectants and sown in rod-rows at Arlington Experiment Farm, Va., March 21, 1925. Infection count—May 27, 1925*

Treatment	Number of striped plants per rod-row from				Total	Average	Approximate per cent infection	Per cent germination
	1922 seed		1923 seed					
	Row 1	Row 2	Row 3	Row 4				
Check	50	69	55	50	224	56	17	83
Uspulun	45	40	36	38	159	40	12	82
Bayer No. 3	26	27	18	15	86	22	7	85
Check	80	65	61	65	271	68	21	82
S.F.A. No. 225	2	2	0	0	4	1	0.3	87
Semesan Jr.	8	4	1	6	19	5	2	83
Check	65	55	80	60	260	65	20	81
Semesan	27	25	0	0	52	13	4	88
Copper stearate	50	55	60	55	220	55	17	86
Check	60	65	39	40	260	65	20	82

SUMMARY

1. From the standpoint of effects on germination it will be noted from table 1 that Corona 620 produced the greatest reduction in the percentage of germination compared with the untreated controls, although S.F.A. No. 175 and Tillantin "C" at 1 per cent for two hours also caused marked injury to germination. The other treatments either did not affect germination or apparently improved it to some extent. Uspulun-treated seed showed the highest average percentage of germination throughout, while next in order came seed treated with Semesan, Bayer Compound, S.F.A. No. 125 and No. 175, Germisan, Tillantin "C" and Corona 620.

2. The Germisan treatments were the only ones that completely eliminated stripe disease, although some of the others reduced it to a very slight trace. Therefore, if stripe-free seed is the aim of the grower, it seems that treatment with Germisan can be recommended, whereas if the only aim of the grower is to reduce the loss due to stripe in the crop grown from badly infected seed, or if seed treatment is to be practised every year, a number of the other disinfectants will serve the purpose equally well. It will be noted that while treatment with Uspulun resulted in the highest percentage of germination (table 1), it also resulted in the highest yield (table 3) while it reduced the stripe disease to a slight trace (table 2). Germisan, on the other hand, while controlling stripe disease perfectly, did not improve the germination of the seed nor materially increase the yield of grain. Semesan, also a chlorophenol mercury compound like Uspulun, was almost equal to Uspulun in its effect on percentage of germination, stripe control, and yield of grain. The same is true of Bayer Compound.

3. According to the data presented, it seems that either Germisan or Uspulun produced the best results, depending on the purpose of the treatment. At any rate, several of the treatments were superior to formaldehyde and copper-sulphate treatments which invariably have been found to produce seed injury.

4. The data presented in table 4 show that the dusts had no apparent effect on the germination of the seed, the treated and untreated seed showing about the same percentage of germination. All of the dusts decreased the amount of stripe to some extent, and a few almost eliminated it. The effectiveness of dust treatments for stripe disease probably depends to a great extent on the amount of moisture in the soil at the time of sowing the seed. Therefore, more work on the relation of soil moisture to the effectiveness of dust treatments is desirable.

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AN IMPROVED METHOD OF ISOLATING PSEUDOMONAS TUMEFACIENS Sm. and Town.

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In connection with our cultural and isolation studies of the organism causing crown gall (*Pseudomonas tumefaciens* Sm. and Town.), it was found that a small amount of sodium taurocholate in dextrose agar inhibits the development of most of the contaminating organisms and seemingly stimulates the growth of *Ps. tumefaciens*. The medium is made as follows: dissolve 15 grams of powdered agar in a double boiler, adding 3 grams of sodium taurocholate and 10 grams of Witte's peptone. After thoroughly digesting these two ingredients, add 20 grams of dextrose and lastly two cc. of a 1–1,000 aqueous solution of crystal violet. Filter twice through cotton and sterilize at 15 pounds pressure for 15 minutes. It is preferable to allow the freshly sterilized agar to cool to 40° C. and to pour the plates immediately, rather than to allow the agar to solidify and then remelt.

The utilization of this medium makes it possible to take large amounts of tissue with comparatively little dilution. Two or three pieces of tissue 10 x 10 x 2 mm. from different parts of the gall are finely chopped in 5 cc. of sterile distilled water in a sterile petri dish. The macerated material is allowed to stand for two to twelve hours to allow the bacteria to diffuse out of the tissue. One to two cubic centimeters of this suspension are placed with a sterile pipette into 50 cc. of melted bile agar, shaken well and used to pour five plates. The poured plates are incubated at 27°–30° C.

The advantages of this method are that less drastic disinfection of the gall tissue is required and a greater quantity of inoculum may be used; the development of the coccus forms and psychrophilic soil bacteria is inhibited by the bile and high temperature of incubation; the growth of the Gram-positive bacteria is inhibited by the crystal violet [Hexamethyl violet (chloride)].

This method does not exclude certain bacterial colonies which have at first a close resemblance to *Ps. tumefaciens* but which later seem to be quite distinct from those of the crown gall organism. *Ps. tumefaciens* is not inhibited, but develops as rapidly as on potato dextrose agar in pure culture.

By this method it is possible to recover *Ps. tumefaciens* from gall tissue, such as apple, much more readily than by using potato dextrose agar and the small amount of inoculum usually employed in dilution plates.

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PHYTOPATHOLOGICAL NOTES

An Improved Method for Isolation of Thielavia basicola. Recently being desirous of isolating the fungus *Thielavia basicola* in pure culture from tobacco roots affected with the Thielavia root rot and remembering the difficulty previously experienced in doing this by the poured plate methods usually employed in such cases and the similar experiences of others who had worked with this fungus, it occurred to me that tobacco seedlings might advantageously be used to assist in securing the pure culture. Accordingly seed of White Burley tobacco was sown quite thickly on blotting paper moistened with sterile water in small glass moist chambers. At the same time tiny bits of the dark brown, infected portions of the diseased tobacco roots above-mentioned were scraped off and scattered among the tobacco seed. The moist chambers were kept at room temperature, and in a few days the tobacco seed germinated. After about 10 days, when the seedlings were ¼ to ⅜ inch tall, it was noted that one had shrivelled at the base and fallen over. On microscopic examination it was found that the root and stem to the very leaves were parasitized by the mycelium of *Thielavia basicola*, and an abundance of spores, both endoconidia and chlamydospores, the former in greater number, were present on the seedling. During the next few days several of these parasitized tobacco seedlings were removed with sterile forceps from the moist chambers and laid on carrot agar plates, and in every instance pure cultures of the fungus were readily secured. The fungus grew out on the agar in most instances without the bacterial contamination usually encountered in making plates direct. At the same time several attempts made by other workers to isolate the fungus from the same material by the usual agar plate method either were unsuccessful or succeeded only after considerable difficulty and delay. The method is so simple, yet so successful, that it may be of assistance to others who have occasion to isolate *Thielavia*, or it may be adapted for use in other, similar cases.—W. W. Gilbert, U. S. Department of Agriculture, Washington, D. C.

Stain Technology. Under the auspices of the Commission on Standardization of Biological Stains, a journal, Stain Technology, has been started under the editorship of H. J. Conn, chairman of the commission, who represents the field of bacteriology. The fields of chemistry, zoology, pathology, and botany are represented respectively by the following associate editors: W. C. Holmes, S. I. Kornhauser, F. B. Mallory and W. R. Taylor. The journal is devoted primarily to the nature and uses of biological stains, including, however, articles dealing with microscopic technic in general when

they have a close bearing on staining procedures. Brief notes of only one or two paragraphs dealing with new staining procedures are welcome.

Authors who have matter for publication along the lines indicated are invited to submit it to the editor at the address: Lock Box 299, Geneva, New York.—H. J. CONN, Chairman, Commission on Standardization of Biological Stains.